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Final Report

Development of mass-trapping methods for codling moth females in disrupted orchards

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Summary

Research aimed to develop an additional non-chemical control option that will complement the use of pheromone-mediated mating disruption (MD), entomopathogenic nematodes, codling moth granulosis virus (CMGV), and the parasitoid wasp *Mastrus ridens* for codling moth management in Australian pome fruit orchards resulted in successful mass-trapping and control of codling moth in pears.

Field trials conducted in 2013-14 at sites in the Goulburn Valley compared the relative attractiveness of 24 combinations of pheromones and host plant volatiles in lures for adult codling moth males and females. The combination that attracted the most codling moths was used in 2014-15 field trials to determine the radius of attraction in orchards treated either with or without MD.

All female moths captured in the traps during 2014-15 were gravid and therefore captured after mating but before laying all of their eggs. Average damage levels to the crop by codling moth were 0.57% under MD and 0.78% without MD. The generally accepted economic threshold is 1% for damage caused by codling moth. The radius of attraction of the traps under MD was found to be approximately 33m for female moths and 43m for male moths. The rate of capture of female moths decreased after 3 weeks exposure of the lures in the field whereas the rate of male capture was relatively consistent throughout the season. This raised the question of whether the decline in capture of female moths was due to depletion of the female population, which could explain the low level of damage to fruit recorded at harvest, or due to depletion of the female attractant in the lure. The estimation of active radius as 33-43m equates to 2-3 traps/ha and a simulation study suggested that 8 traps/ha were required to accurately determine the spatial density of the codling moth population. Active radius is a trap characteristic independent of size and spatial arrangement of the pest population, whereas the number of traps required for reliable estimation of the mean is dependent on the spatial arrangement of the pest population. Some degree of overlap of trap active spaces would eliminate gaps in coverage and therefore the optimal number of traps/ha will be between two and eight for mass-trapping.

In 2015-16 the experimental design was modified to simultaneously explore issues related to lure longevity and efficacy of mass-trapping while still adhering to the aim of the original methodology. Damage recorded at harvest 2016 was 0.083%. Although the rates of female capture in traps baited with lures changed at 3 and 6 weekly intervals were similar throughout the season and both were greater than for traps in which the lures were not changed at all during the season, the means were not statistically significant. Variograms fitted to male, female, and total moth capture resulted in estimation of active radius to be 35m, 32m, and 30m respectively, which are close to the range determined in 2014-15.

The project results provide proof of concept for application of mass-trapping methods to enable non-insecticidal control of codling moth populations in orchards under mating disruption. Damage levels attributed to codling moth were reduced from 0.57%, at the end of the first year of mass-trapping, to 0.083% at the end of the second year. This is in line with the forecast outcome of less than 0.1% crop damage. Further work is required to determine the optimal spatial arrangement for the traps, size of the trapping surface, and to optimize the release rates of the volatile components of the lures to ensure cost-effectiveness.

Keywords

Pome fruit, Integrated Pest Management; codling moth, mass-trapping, *Cydia pomonella*

Introduction

Codling moth (CM) *Cydia pomonella* L. (Lepidoptera: Tortricidae) is the most serious pest of pome fruit worldwide and the most damaging pest of commercial apple, pear, quince and nashi orchards in Australia. Widely distributed in all Australian states except Western Australia and Northern Territory (Poole 2004), *C. pomonella* was controlled by multiple applications of organophosphate insecticides but resistance and health concerns resulted in these being phased out (Thwaite et al. 1993). Newer pesticides with lower human toxicity are more specific but not as effective. Application of sex pheromone mediated mating disruption (MD) can be an effective alternative to the use of pesticides for control of low-moderate population levels of codling moth, can significantly reduce the number of insecticide sprays, and have been the key-stone of IPM in the fruit growing areas of Victoria (Vickers and Rothschild 1991, Brown and Il'ichev 2000, Williams and Il'ichev, 2003). Control of moderate-to-high population densities is more problematic (Vickers et al. 1998) and several consecutive seasons of area-wide MD treatments are needed to control higher pest population levels (projects FR-01008, FR-04009, MT-07028). If the female population density is sufficiently high compared to the distribution density of the MD dispensers some males will find females instead of dispensers, and mating will occur. This effect is called competitive attraction and the size and distribution of the female moth population determines the spatial density of MD dispensers required to out-compete the females. Hand applied MD dispensers are too expensive to distribute in high enough "point-source" density to control high populations of codling moth (Gut et al. 2004) and sprayable formulations that provide high point source density do not emit pheromone for long and require regular re-application (Gut and Brunner 1998, Stelinski et al. 2007).

The pear-derived kairomone, ethyl (E, Z)-2, 4-decadienoate ("DA" or "pear ester") is a species-specific attractant, for male and female codling moths, used for monitoring CM females in apple orchards treated with MD (Light et al. 2001, Il'ichev et al. 2002, Knight and Light 2005, Knight et al. 2005). Field trials of DA baited traps demonstrated that the ratio of mated: virgin females caught in DA traps increased as the moth population density increased (Il'ichev 2004, Thwaite et al. 2004, Il'ichev et al. 2009). Other field trials demonstrated that combining DA or (E)-4,8-Dimethyl-1,3,7-nonatriene (DMNT) loaded in septa lures with separate vials containing acetic acid significantly increased both male and female moth catch compared with DA, DMNT or acetic acid alone. However, traps baited with DA plus acetic acid caught significantly more male and female moths than traps with DMNT plus acetic acid (Knight et al. 2011)

Female pupae emit sex pheromone prior to emergence of the adult female moth and males emerging close by can bypass the normal searching behaviour, visibly detect the female moth and exhibit courtship behaviour leading to copulation (Duthie et al. 2003). MD is not able to compete with such behaviour. It is therefore important to develop an independent mechanism to reduce the female moth population before they lay eggs.

Mass-trapping using CM sex pheromone baited traps to capture male moths was successful against isolated, low-density populations. However, this failed to control higher population densities due to competition from high populations of calling females, high cost of the number of traps used per hectare, and presence of un-trapped polygamous CM males able to mate with several females (Thwaite and Madsen, 1983).

There has been renewed interest in the development of lures containing DA, and specifically research aimed at increasing DA attractiveness for CM mated females through the addition of synergistic host-plant volatiles (Vallat and Dorn 2005, Witzgall et al. 2001, Yang et al. 2004). More powerful attractants will help fill the void between the use of MD and the use of pesticides to kill eggs and larvae.

The objectives of the study reported here were:

1. To assess the effectiveness of putative synergists in mixtures with pear ester and/or codlemone for attracting codling moths
2. To investigate the active space of traps containing the best performing combination lure identified from objective 1 above.
3. To determine the optimal spatial density of the traps for use in mass-trapping of codling moths in pear orchards

Methodology

2013-14

Two field sites were established in the Goulburn Valley, Victoria, to assess the relative attractiveness of various combinations of pheromones and host plant volatiles in lures for male and female codling moths. A block of codling moth infested Corella pears surrounded by Nashi at Bunbartha was the trial site for 6 lure types (combinations) replicated 4 times in a Randomized Complete Block Design. Delta traps baited with individual lures were placed in the top meter of the tree canopy on 10 October 2013 and monitored weekly.

A large orchard in Ardmona containing Packham pears with a history of codling moth infestation was selected as a trial site for a larger experiment using 24 lure types (combinations) replicated 4 times in a Randomised Incomplete Block Design.

In both experimental sites, all traps were monitored weekly and all captured moths were counted before being removed for sexing by examining genitalia under the dissecting microscope. For identification of female mating status, the abdomen of CM females was dissected and bursa copulatrix inspected for presence of spermatophores.

2014-15

A block of pears cv Packham's Triumph in an orchard at Ardmona, Victoria, Australia, with a history of damage by codling moth, and treatment with pheromone mediated mating disruption, was selected as the study site. Tree rows were 5.9 m apart and trees within the rows were spaced approximately 5.1 m apart. The experimental plot was approximately 2.6 ha in size. One delta trap baited with the test lure (codlemone + pear ester + a confidential host plant volatile) was placed on a branch within the top metre in the outer edge of the canopy in every 2nd tree (starting 3 trees in from the western end of the row) in every 2nd row (starting 3 rows in from the northern side of the block) until 14 traps had been placed in each of 8 trapped rows. This resulted in a grid of 112 traps in which each trap was approximately 11.8 m away from its nearest neighbour across a row or 10.2 m within rows.

Another block of the same pear variety, located about 145 m to the south of the first block, with a similar history of codling moth damage, with trees the same age and laid out the same way as in the first block, was used to compare active space of the traps when mating disruption pheromone was not present. Traps were established using the same protocol as per the first block.

The location of all traps was recorded as GPS coordinates. Lures in the traps were not periodically changed (normal practice would be to change lures every 6-8 weeks) because we were interested in testing longevity of the lures as well as attractive radius. Traps were inspected each week for the presence of moths. All trapped codling moths were counted, collected into vials and transported to the laboratory for determination of gender, and mating status of females.

The cumulative catch in each trap and the GPS referenced location of each trap, was used in Vesper 1.6 (Australian Centre for Precision Agriculture, The University of Sydney, NSW, Australia) to produce a spatial prediction of the codling moth population density in the orchard at each sampling date. This enabled estimation of active radius.

To determine a sample size (number of traps) that adequately estimates the average catch in the population of 112 traps, we generated 200 random samples of 2-15, 20, 25, 30, 35, 40, 45, and 50 traps using simple random sampling without replacement. The arithmetic mean of each of these 200 random samples was then computed. The distribution of these 200 mean values for different sample sizes was summarized using box plots for female, male and total catch. Box plots were also produced for the distribution of Coefficient of Variation (CV) from the same data.

To explore the extent of spatial dependence between traps we fitted variograms to the spatial data for moth catches using models with 200 lags, lag tolerance = 0.5, maximum distance = 100m, and weighting = number of pairs. The point of inflexion, or the start of a sill, in the variograms indicates the distance beyond which traps cease to be spatially dependent. There were high numbers of zero catches throughout the season due to spatial variation in population density, so the data were log(x+1) transformed before fitting a variogram using a linear-with-sill model in Vesper 1.6. The active space of a trap that relies on a plume of volatiles for attraction is represented in two dimensions as the area of a circle centred on the trap. The point at which two traps cease interfering with each other is the point at which the two circumferences meet but do not overlap. The length of the line drawn between the traps, that passes through this point, is the separation distance indicated by the variogram. If the two traps are identical then the length of the line between the two traps is twice the radius of the active space.

Fruit damage assessments on 100 trees (100 fruits per tree) within each block with and without MD (ie 200 trees and 20,000 fruits in total) were conducted close to the end of the growing season, but just before commercial harvest.

2015-16

The aims of the work conducted 2015-16 were to assess the field life of the lure and to determine potential impacts on efficacy of mass trapping. The same pear blocks at Ardmona, used in previous seasons, were selected as the study site. One delta trap baited with the test lure (codlemone + pear ester + a confidential host plant volatile) was placed on a branch within the top meter in the outer edge of the canopy of each of 4 trees per plot, with 3 plots (treatments) per experimental block, and 10 blocks in total. Treatments allocated to plots were based on frequency of changing the lures. Treatments were (1) no change of lure; (2) lure changed every 6 weeks; (3) lure changed every 3 weeks.

The location of all traps was recorded as GPS coordinates so that spatial analysis could be used to confirm the active radius calculations from the previous season. Traps were inspected each week for presence of moths. All trapped codling moths were counted and then collected into vials for transport to the laboratory to determine gender by examining genitalia under the dissecting microscope. Data were analyzed using RCBD-based repeated measures ANOVA. Variograms were used to determine active space, as per 2014-15 above.

Fruit damage was conducted by inspecting 200 fruit from the central tree in each plot.

Outputs

Output 1: *A report on demonstration of proof-of-concept for application of mass-trapping methods for non-insecticidal control of codling moth populations in orchards under mating disruption.*

This final report provides the proof of concept. The first year of the project determined the most promising combination of volatiles for use in lures. The second year investigated spatial aspects and efficacy of the lures, and determined the active space of traps baited with the lures but also raised questions about the longevity of the lures. Year three investigated lure longevity and confirmed the active space of the traps while demonstrating proof of concept of mass-trapping by achieving very low levels of fruit damage (0.083%).

DEDJTR is developing a project proposal for the next stage of the work. This is for discussion with Horticulture Innovation Australia, industry, and potential commercial partners.

Output 2: *A report on key issues related to integration of codling moth female mass-trapping and improved monitoring in pheromone disrupted orchards, into pome fruit orchard IPM programs.*

Mass-trapping using the lures developed in the project will reduce numbers of both male and female moths. This will enhance the potential for pheromone-mediated mating disruption to maintain codling moth populations, and resultant fruit damage, at low levels. Mass-trapping will complement biological control of codling moth by the recently released parasitoid wasp *Mastrus ridens* through reducing the risk of exposure to pesticides that may be toxic to the wasp (Appendix 4).

Output 3: *Articles prepared for publication in the industry magazine to provide awareness of project progress (subject to project confidentiality).*

- The results of the project have been included in several workshop presentations to growers and industry service providers:
- Presentation to the HIN (Horticulture Industry Network) to create awareness with industry IDOs in the first 3 months of the project.
- Cooperating growers were kept informed via regular updates of the trapping results in their orchards.
- The project was outlined to growers, consultants, APAL staff and other service providers at the APAL Speed Updating Forum 2013.
- Fruit Growers Victoria meeting in Mooroopna on 12 May 2014.
- An article summarizing progress was submitted to HAL on 6 May 2014 for inclusion in the Apple and Pear IAC Annual Report.
- AgriLink Agronomy Forum in Lilydale on 29 May 2014.

- Regional Stone and Pome Fruit Research and Development Forum organized by the Horticulture Centre of Excellence, HAL, Summerfruit Australia, and APAL on 22 October 2014 in lieu of the APAL Conference that did not occur in 2014.
- Presentations were also made at the APAL Speed Updating Forum and Fruit Growers Victoria end of season meetings in June 2015.
- Regional Innovation Forum "Delivering innovation through the horticulture supply chain", 18th May 2016

Output 4: *Draft scientific papers and conference presentations (including APAL conference in 2016) to be produced without revealing confidential results after the final report has been accepted by Horticulture Innovation Australia.*

The 2016 APAL conference does not provide for presentation of the results however the project leader will be presenting at the "Pome Zone" section of the conference. The presentation will include the results of this project as well as progress in the IPM component of PIPS 2. A presentation including results from this project is being developed by Dr Il'ichev for the International Society of Chemical Ecologists scientific conference in Brazil, June 2016, but at time of writing this report was still in development.

At least two scientific papers are planned to be produced after the final report has been accepted by Horticulture Innovation Australia. One relates to proof of concept of mass-trapping. The other relates to improvements in the active space of traps, utilizing the combination lure, in orchards under mating disruption. These are not expected to be submitted to journals until late 2016 and approval to publish will be sought from Horticulture Innovation Australia prior to submission.

Outcomes

Because the project was designed as a proof of concept, with potential commercial-in-confidence results, it is too early to have achieved outcomes such as registration, adoption and widespread reduction in codling moth populations. Assuming that proof of concept is accepted, then approval of the next phase of the work (i.e. fine tuning of the lure release rate characteristics and spatial distribution of the traps) should lead to commercial development.

The project has demonstrated how in pear orchards treated with pheromone-mediated mating disruption:

- A combination of codlemone plus pear ester and another confidential plant volatile outperformed 23 other combinations of volatiles, including codlemone plus pear ester and acetic acid, in trapping male and female codling moths.
- The active space of delta traps baited with the best performing lure is between 35-43 m for trapping male, and 32-33 m for trapping female, codling moths.
- Delta traps baited with the best performing lure could be used at 8 traps/ ha to produce reliable estimates of codling moth population density.
- Damage levels attributed to codling moth were reduced from 0.57%, at the end of the first

year of mass-trapping, to 0.083% at the end of the second year. This is in line with the forecast outcome of less than 0.1% crop damage, that was provided in the project submission.

Three potential commercial partners have been identified to date:

- The project team has been approached by a trap manufacturer in China who has a mini trap that may be a cheap alternative to the full sized delta traps used in the experiment. The impact of trap size on active space and trapping efficiency would need to be explored as part of the commercialization process. The manufacturer has an interest in semio-chemicals and could also produce lures.
- The lures used in the experiment were produced under contract by a manufacturer in Costa Rica who has the technology and expertise to tailor release rates for individual components. Further work is required to determine optimum release rates for each component before seeking registration.
- Another manufacturer/pest management equipment supplier in the USA is also interested in collaborating in the commercialization process.

Other outcomes likely to flow in the future from, but not under direct control of, the project include:

- Enhanced survival of biocontrol agents (such as *Mastrus* against codling moth), *Aphelinus mali* (against woolly apple aphid), lacewings and syrphid flies that prey on aphids, predatory mites and *Stethorus* beetles that prey on phytophagous mites, and the range of fly and wasp parasitoids that attack lightbrown apple moth) as a result of reduced pesticide applications against codling moth.
- Prolonged life of existing narrow-spectrum pesticides due to improved adherence to resistance management protocols made feasible by reduced reliance of multiple pesticide applications to control codling moth.
- Generation of new projects targeting mass-trapping of other insect pests.

Evaluation and Discussion

2013-14

Lures containing codlemone alone only attract male codling moths. Addition of pear ester (DA) to codlemone (CM)-baited traps in orchards under mating disruption enhances capture of males and also attracts females. Recent research suggests that addition of acetic acid to traps baited with CM/DA further increases capture of moths but this is not supported by our results for both sexes combined. The seven best performing lures based on total catch (both sexes combined) at Ardmona were, in descending order CM/DA/T5, CM/DA, CM/DA/AA/T4, CM/DA/AA, CM/DA/AA/T5, CM/DA/T4, and AA/DA although the numbers caught in traps baited with these lures were not significantly different. Traps baited with CM/DA/T5 caught significantly more moths (both sexes combined) than the other lures, including CM/DA, at Bunbartha. This may indicate a varietal influence between Packham and Corella pear volatiles.

The best performing lure for capturing female codling moths at Ardmona was AA/DA with AA/DA significantly more attractive than the next best performers CM/DA/AA/T4, CM/DA/AA/T5, CM/DA/AA, DA/AA/T5, and CM/DA/AA (none of which were significantly more attractive than each other). In the Bunbartha experiment there were no significant differences between the two best performers AA/DA and DA/AA/T5 but they were both significantly different to DA/AA/T4 which in turn was significantly more attractive than CM/DA/T5, CM/DA/T4, and CM/DA. Combining AA in a single lure with any of the other test components is difficult due to chemical reactions and AA is therefore supplied separately, which increases cost and handling issues.

The data collected in the 2013-14 season indicated that CM/DA/T5 could be a useful combination for not only mass trapping both sexes of codling moth in orchards under pheromone-mediated mating disruption, but also for improved monitoring of moth populations since the normal pheromone baited traps are notoriously unreliable in such orchards (Appendix 1).

2014-15

Volatile attractants create an active space around a source point. The active space is the area in which the semiochemical concentration is above a behavioural threshold eliciting orientation by the moth towards a source point. The number of traps/ha required to reliably monitor a moth population in an orchard depends on the active space of the trap, the behaviour of the target moth species, and spatial distribution of the moth population in the orchard. Traps using combinations of semiochemicals could be useful tools for both monitoring and/or mass-trapping pest insects, especially in orchards where pheromone-based mating disruption (MD) is being used as a pest management tool and inadvertently decreases the efficacy of normal pheromone traps as monitoring tools.

The rate of capture of female moths in both MD and non-MD treated blocks decreased after 3 weeks of trapping whereas the rate of male capture was relatively consistent throughout the season. More females than males were caught in the MD treated block in the 2nd and 3rd weeks, suggesting that either the female population was rapidly depleted in the first 3-4 weeks or the new female attractant component of the lure was depleted after 3 weeks and females continued to be captured throughout the rest of the season as a result of continued emission of the pear ester component of the lure. A similar result was observed in the non-MD treated block although in that case the number of females caught never exceeded the number of males. Damage levels to the crop by codling moth were 0.57% for the MD treated block and 0.78% in the non-MD block. Such low levels of damage suggest that the mass trapping may have significantly reduced the moth populations. If the lure components had depleted then more frequent replacement of the lures, or a change of formulation to ensure better longevity, should enhance female capture.

Variograms for the early part of the season, when the populations were high, demonstrated a sill at 67 m for females and 86 m for males, which translate to active radius of 33.5 m and 43 m respectively. The variograms for the control plot (not under mating disruption) did not yield a relationship for female moths but the active radius for male moths averaged 29.5 m which is considerably shorter than the 43 m active radius for male trapping in the MD treated block.

The mean values of population estimates derived from the simulation study indicated that at least 20 traps were required to obtain consistent results. Scaling down from the 2.6 ha plot to one hectare yields 8 traps/ha as a minimum in blocks treated with mating disruption. At that trapping density, and if the traps are evenly spaced, each trap would service 10000 $m^2/8 = 1250$ m^2 of non-overlapping space. Given that most orchard blocks are either square or rectangular in shape, and trapping theory tends to utilize a circular shape for active space of a trap, it is reasonable to assume that there would be some overlap of active spaces in deploying 8 traps. A square of area equal to 1250 m^2 would have sides of 35m and a diagonal distance of 49.5 m, which means that an enclosing circle that just touches each of the corners of the square would have a radius of 24.75 m. A circle of radius 33.5 m (active radius, under MD, for trapping females) would enclose a square with sides 47.4 m long. The difference only equates to 2 tree spacings, which is probably within the margin of error for the techniques used but could also suggest that it is necessary to have some degree of overlap of trap active spaces to get reliable estimates of the moth population mean, possibly because it improves the ability to detect hotspots.

All female moths captured were gravid. Average damage levels to the crop by codling moth were 0.57% for the MD treated block and 0.78% in the non-MD block (Appendix 2).

2015-16

In 2015-16 the experimental design was modified from the original completely randomised design with 3 treatments and 5 replications, to a randomised complete block design with 3 treatments and 10 replications so that we could simultaneously explore issues related to lure longevity and efficacy of mass-trapping while still adhering to the aim of the original methodology.

Discussions were held with the lure supplier with respect to possible modification of the lure body to accommodate different release rates of the individual attractants. The supplier was confident that could be achieved but completion of the 2015-16 season's work was required in order to specify the desired release rates for individual components. This also limited the value of discussing registration with APVMA because they require information on components, release rates, and efficacy of the final version of the lure.

There were no statistically significant differences between treatments (F Prob: 0.620 males, 0.331 females) or treatment X week interactions (F Prob: 0.079 males, 0.060 females) but there was a significant effect of week (F Prob: <0.001 in both cases) in terms of cumulative numbers of moths captured in traps that did not have the lures changed for the season (treatment 1), had lures changed every 6 weeks (treatment 2), or had lures changed every 3 weeks (treatment 3). However, in the last four weeks of trapping comparison of the means using LSD 5% indicated that treatment 2 caught significantly more female moths than treatment 1 but the results for treatment 2 vs treatment 3, and treatment 3 vs treatment 1 were not significantly different. This is difficult to explain in terms of lure longevity since treatment 3 had the most frequent changes of lure and was expected to perform better than the other two treatments if longevity was the issue, as suspected at the end of 2014-15. The situation was reversed with capture of male moths, with treatment 3 capturing significantly more moths than treatment 1 and captures in treatment 2 not being significantly different to those in treatment 1 or treatment 3. The results suggest that the lures last for at least 6 weeks.

Variograms fitted to total male capture, total female capture, and total moths (both sexes combined) indicated separation distances of 69 m, 63 m, and 59 m respectively, which translate to active radius of 34.5 m, 31.5 m, and 29.5 m for males, females and total respectively. These basically confirm the results of 2014-15.

Fruit damage was 0.083% at harvest and is in line with the forecast outcome of less than 0.1% crop damage, that was provided in the project submission (Appendix 3).

In summary, the project met its objectives by:

- Identifying the most promising combination of volatiles for use in lures (Appendix 1)
- Determining the active space of traps baited with the lures (Appendix 2)
- Confirming suitable longevity of the lures while demonstrating proof of concept of mass-trapping by achieving very low levels of fruit damage (0.083%) (Appendix 3).

Recommendations

The next stage of the work will move towards making mass-trapping a practical reality by resolving the potential conflict between theoretical best practice and practical reality. The proof of concept study used 43 traps/ha to generate data that suggest between 3 and 8 traps/ha may be sufficient in pear orchards. Further work is required to determine the optimal spatial geometric arrangement of traps for mass trapping, and if similar results can be obtained in apple orchards.

If relatively small numbers of traps/ha are all that is required then it will not be necessary to develop mini traps, but it is essential to develop a lure that can optimize the individual component release rates in order to ensure that the ratio of components released over time remains stable and effective. The current supplier of the lures has this capability and is interested in collaborating to achieve such an output.

We recommend that Horticulture Innovation Australia and the Government of Victoria (through the Department of Economic Development, Jobs, Transport and Resources) evaluate the IP in the project to determine if it is appropriate to patent or otherwise protect and commercialise.

Scientific Refereed Publications

None to report at this stage because of potential commercial–in-confidence content.

Once this report has been approved draft papers will be developed in a way that does not negate prospects of commercialization.

Intellectual Property/Commercialisation

The potential commercial IP generated by the project is the identity of the additional chemical component in the lure. Other IP generated by the project includes the "know-how" behind the successful mass-trapping of codling moth in pears. Pre-existing IP brought to the project by the project team includes knowledge of trapping systems; understanding of codling moth biology, phenology and chemical ecology; expertise in project design and analysis; and application of basic principles to achieve practical outcomes.

The combinations of components used in the lures is commercial-in-confidence, as per the research contract, and all communication activities conducted by the project team have used codes to protect the identity of the components that are subject to commercial-in-confidence.

Production of the experimental lures and discussion of possible modifications was covered by an agreement with the manufacturer to protect IP.

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Appendices

Appendix 1: Relative efficacy of various lures for trapping codling moth females, males, and both sexes combined

Appendix 2: Active space, and required number, of traps baited with a combination of semiochemicals for mass-trapping of codling moth in pear orchards

Appendix 3: Mass-trapping of codling moth in orchards

Appendix 4: Integrating mass-trapping of codling moth into orchard pest management strategies

Appendix 1:

Relative efficacy of various lures for trapping codling moth females, males, and both sexes combined

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Introduction

Codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae) (CM) is a serious pest of pome fruit worldwide and the most damaging pest of commercial apple, pear, quince and nashi (Asian pears) orchards in Australia. Codling moth is widely distributed in Australian states except the Northern Territory and Western Australia, which is considered to be free of CM after a successful eradication program (Poole 2004). Uncontrolled CM moth larvae can destroy significant amounts of the crop (Geier 1963). Although Australian growers have generally associated codling moth damage with pome fruit it also infests walnuts and in several European countries, Chile, Argentina and the USA it has been recorded from stone fruits (including peaches, apricots, and cherries). Resistance to organo-chlorine, organo-phosphate, and carbamate pesticides and some insect growth regulators is widespread. Review and withdrawal of synthetic pesticide registrations increases the selection pressure on any new pesticides and resistance management strategies based on limited spray applications are common, but their adoption is hindered by lack of suitable alternative control measures.

Mastrus ridens attacks the pre-pupal final instar codling moth larvae within their cocoons. *M. ridens* is being investigated in the current PIPS IPDM program (HAL Project AP09031), and entomopathogenic nematodes are now commercially available in Australia. Both will reduce the number of moths emerging in spring. However, codling moth larvae utilise an aggregation pheromone that results in larvae hibernating close to each other. In spring the female pupae emit pheromone that "arrests" nearby male moths that can then mate with the virgin female moth when she emerges from the pupa. This is one of the reasons that MD is less effective against high population densities of codling moth.

Project MT12000: "Development of mass-trapping methods for codling moth females in disrupted orchards" aims to deliver an additional non-pesticide based control option that will complement the use of mating disruption (MD), entomopathogenic nematodes, codling moth granulosis virus (CMGV), and the newly introduced parasitic wasp *Mastrus ridens* for codling moth management in Australian pome fruit orchards. The project builds on work conducted on trapping female codling moths under HAL project MT07028, and recent discoveries overseas, to develop and test reliable mass-trapping methods that can be integrated with biological control and mating disruption for control of codling moth.

There are substantial benefits for the apple, pear and nashi fruit industries from this approach. The gross value of pome fruit production (excluding nashi) in Australia in 2008 was estimated at $450 million. Victoria (217,300 tonnes) is the major pome fruit production region in Australia with 113,300 tonnes of pear and 104,000 tonnes of apple produced in 2008. Left untreated, codling moth can cause losses exceeding 30% of the crop and even complete crop loss although such severe attack is not often seen in commercial orchards because of the use of pesticides and semiochemicals such as pheromone mediated mating disruption. An improvement of packout by even 1% would be worth $4.5M annually.

Mass-trapping of mated female codling moths before they lay eggs will reduce the number of eggs laid, and larvae produced, by each generation. Larvae hatching from any eggs laid will be targeted by either CMGV, conventional pesticides, or by *Mastrus ridens*, reducing the population to densities more easily controlled by MD in subsequent generations.

The first phase of the project involves field screening experiments comparing responses of CM females to various candidate mixtures of volatile compounds to determine the most effective mixtures for use in further field studies as lures. This report meets the requirement of Milestone 103, a stop-go milestone. If the recommendation to continue the research is accepted then studies to determine the active radius of attraction of the best performing lure in a standard trap in the field will identify the optimal spatial density of the trap and lure for further mass trapping experiments. This project will demonstrate proof of concept of mass-trapping of mated CM females that will be measured by reduction in moth populations during the growing season and fruit damage at pre-harvest time.

Materials and methods

Two experiments were established to investigate potential for a range of host-plant volatiles (HPVs) to act synergistically or alone as attractants for codling moth. The HPVs DA (pear ester or ethyl *(E, Z)*-2, 4-decadienoate) and AA (acetic acid) are already in the common domain as synergists of CM (codlemone *(E, E)*-8,10-dodecadienol) used in lures. Two additional HPVs are not named in this report but are commercial-in-confidence and coded T4 and T5.

The first experiment was established in a pear orchard at Ardmona, west of Shepparton. Twenty four odour mixtures, incorporated into lures, were placed in Delta traps. Traps were suspended on bamboo poles such that they hung in the top meter of the tree canopy, and were used to monitor codling moth in the experimental plots. Trees with traps were about 24 m apart both within tree rows and across rows in the orchard blocks. Each mixture was designated as a treatment and assigned to a trap using a randomized complete block design. Four orchard blocks of Packham pears were used as statistical blocks each containing a complete set of treatments, allowing for 24 treatments with 4 replications. All four orchard blocks were treated with hand applied mating disruption pheromone dispensers.

Traps were inspected each week starting one week after the traps were set (30-31 November 2013) and finishing when the crop was harvested (13 February 2014). Codling moths caught in the traps were counted, removed and placed in alcohol-filled specimen tubes labelled with treatment code and replication number for transport to the DEPI Tatura Invertebrate Sciences laboratory for determination of gender. The presence of a spermatophore in dissected female moths was used to confirm mating status.

The second experiment was established at Bunbartha, north of Shepparton, in a predominantly Nashi orchard. The orchard had two blocks of Corella pears, with a history of codling moth damage, that were used to test 4 replications of 6 lure combinations in a randomized complete block design. The trees in the Corella blocks were approximately 2 m high and grown on a low trellis with rows 4 m apart. Delta traps (as per the first experiment) were hung at about 1.6 m above the ground and placed 24 m apart down the rows and 24 m apart across the rows. Each row containing traps had a full complement of 6 treatments. The blocks were treated with hand applied mating disruption pheromone dispensers. Traps were placed 11 October 2013 and checked each week, as per the first experiment, until 26 February 2014.

Cumulative number of moths captured over time during the experiments was plotted to present trends for each treatment (lure). The trap catch data were analyzed using a linear model that included lure, date, and lure x date interaction as fixed effect terms and replicate as a random effect term. The residuals arose from dates nested within traps. As the residuals from repeated measurements on a trap on different dates could be correlated, this auto-correlation was accounted for by a first order auto-regressive process. All analyses were conducted in GenStat 16.0 software using residual maximum likelihood (ReML) after transforming the trap catch data y to log(y + 1), as this more reasonably satisfied the ReML assumptions of normality and constant residual variance.

Results

The untransformed average cumulative number of moths (both sexes combined, and females only) captured over time for each treatment is given in Figures 1-2 for the Ardmona experiment and Figures 3-4 for the Bunbartha experiment. Tables 1-4 present the results of the statistical analysis, using the transformed data.

Figure 1: Cumulative catch of codling moth (both sexes combined) averaged over 4 replications for each treatment (combination of volatiles) at Ardmona. AA= acetic acid; CM= codlemone; DA= pear ester; T4 and T5 are coded due to commercial-in-confidence.

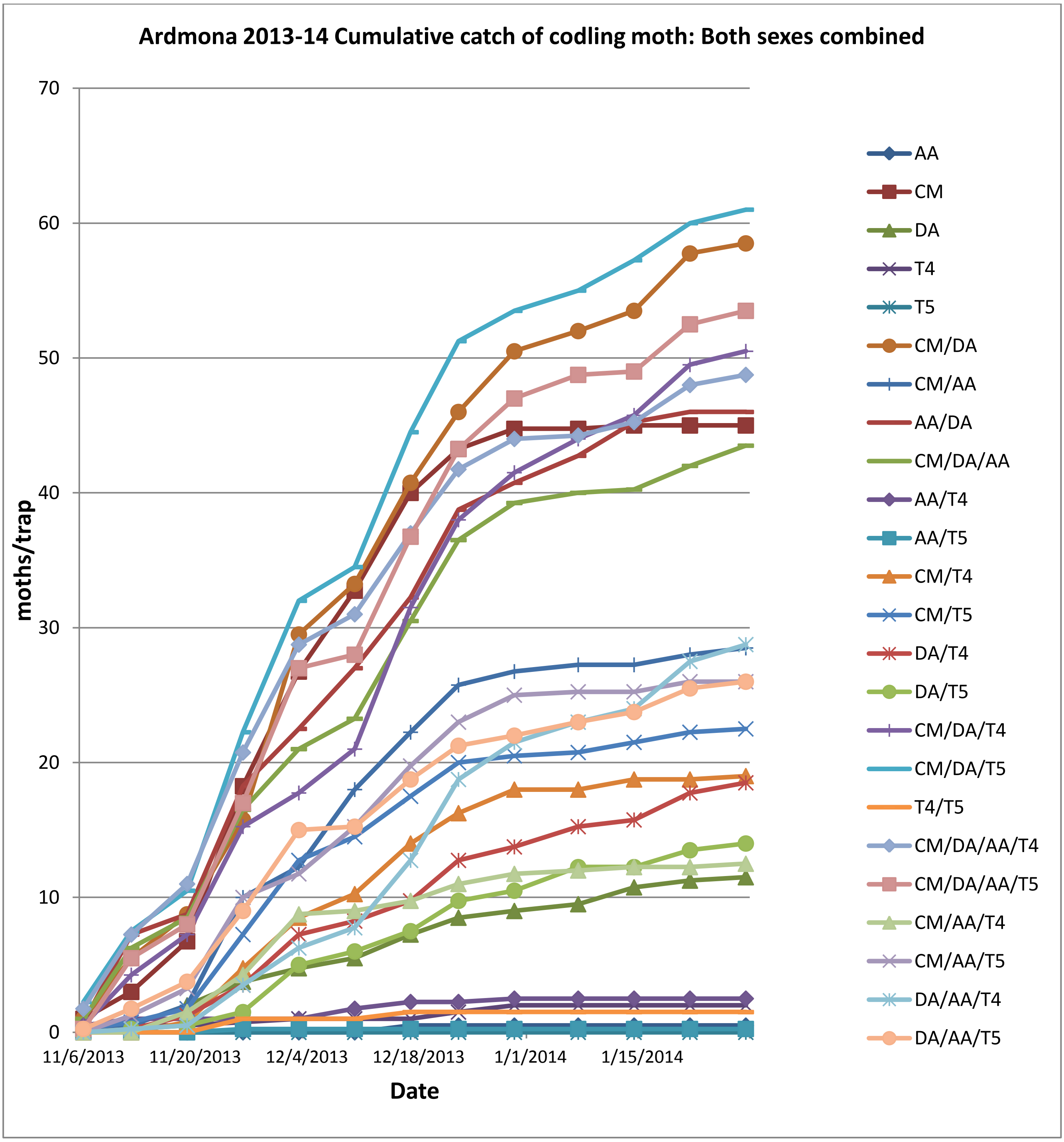

Table 1. ReML estimates of mean log(total catch +1) for Ardmona. Only the seven best performing lures based on the mean catch are annotated by a letter following the mean, with the same letter indicating that the means are not significantly different. There were significant effects of Date, Lure, and Lure x Date interaction. F Prob (Date) < 0.001; F Prob (Lure) < 0.001; F Prob (Lure x Date) < 0.001. SEd(Date) = 0.03465; SEd(Lure) = 0.05311; SEd (Lure x Date) = 0.1697. AA= acetic acid; CM= codlemone; DA= pear ester; T4 and T5 are coded due to commercial-in-confidence.

Lure	6-Nov-13	12-Nov-13	19-Nov-13	26-Nov-13	3-Dec-13	10-Dec-13	17-Dec-13	23-Dec-13	30-Dec-13	7-Jan-14	14-Jan-14	21-Jan-14	28-Jan-14	5-Feb-14	12-Feb-14	Mean
AA	0.000	0.000	0.000	0.000	0.000	0.000	0.119	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008
AA/DA	0.151	0.795	0.294	1.015	0.683	0.624	0.676	0.834	0.401	0.345	0.496	0.195	0.000	0.075	0.411	0.466[a]
AA/T5	0.000	0.000	0.000	0.075	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005
AA/T4	0.151	0.151	0.000	0.000	0.000	0.151	0.119	0.000	0.075	0.000	0.000	0.000	0.000	0.000	0.000	0.043
CM	0.226	0.350	0.564	1.007	0.959	0.809	0.896	0.527	0.325	0.000	0.075	0.000	0.000	0.151	0.151	0.403
CM/AA	0.000	0.119	0.345	0.750	0.464	0.769	0.580	0.508	0.270	0.151	0.000	0.151	0.119	0.075	0.075	0.292
CM/AA/T5	0.000	0.345	0.452	0.831	0.345	0.639	0.714	0.476	0.420	0.075	0.000	0.195	0.000	0.119	0.195	0.320
CM/AA/T4	0.000	0.000	0.270	0.552	0.619	0.075	0.195	0.270	0.195	0.075	0.075	0.000	0.075	0.075	0.075	0.170
CM/DA	0.226	0.675	0.581	0.826	1.113	0.628	0.861	0.714	0.683	0.294	0.345	0.548	0.195	0.075	0.270	0.536[a]
CM/DA/AA	0.270	0.765	0.437	0.850	0.707	0.496	0.896	0.843	0.496	0.195	0.075	0.433	0.325	0.226	0.581	0.506[a]
CM/DA/AA/T5	0.075	0.558	0.464	0.928	0.811	0.239	0.822	0.834	0.540	0.376	0.075	0.556	0.270	0.226	0.615	0.493[a]
CM/DA/AA/T4	0.369	0.705	0.632	0.915	0.940	0.406	0.754	0.721	0.489	0.075	0.226	0.401	0.195	0.151	0.725	0.514[a]
CM/DA/T5	0.433	0.712	0.477	1.058	0.960	0.520	0.874	0.855	0.476	0.345	0.420	0.500	0.239	0.151	0.301	0.555[a]
CM/DA/T4	0.151	0.571	0.445	0.770	0.445	0.420	1.010	0.633	0.533	0.376	0.314	0.551	0.226	0.270	0.301	0.468[a]
CM/T5	0.075	0.119	0.270	0.766	0.670	0.369	0.505	0.389	0.119	0.075	0.195	0.151	0.075	0.151	0.239	0.278
CM/T4	0.000	0.000	0.195	0.676	0.588	0.420	0.556	0.464	0.369	0.000	0.151	0.000	0.075	0.358	0.151	0.267
DA	0.075	0.075	0.294	0.389	0.270	0.195	0.345	0.301	0.151	0.119	0.250	0.119	0.075	0.000	0.075	0.182
DA/AA/T5	0.075	0.294	0.464	0.782	0.834	0.075	0.632	0.437	0.195	0.270	0.195	0.401	0.151	0.226	0.639	0.378
DA/AA/T4	0.000	0.075	0.075	0.556	0.489	0.345	0.646	0.692	0.489	0.358	0.270	0.639	0.195	0.151	0.469	0.363
DA/T5	0.000	0.075	0.075	0.270	0.564	0.270	0.345	0.489	0.195	0.369	0.000	0.314	0.119	0.000	0.000	0.206
DA/T4	0.000	0.075	0.270	0.445	0.520	0.270	0.270	0.512	0.226	0.389	0.151	0.331	0.195	0.075	0.151	0.259
T5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.075	0.005
T4	0.075	0.075	0.000	0.075	0.075	0.000	0.000	0.119	0.119	0.000	0.000	0.000	0.000	0.000	0.151	0.046
T4/T5	0.000	0.000	0.000	0.175	0.000	0.000	0.119	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020
Mean	0.098	0.272	0.275	0.571	0.502	0.322	0.497	0.442	0.282	0.162	0.138	0.228	0.105	0.106	0.235	

Figure 2: Cumulative catch of codling moth females averaged over 4 replications for each treatment (combination of volatiles) at Ardmona. AA= acetic acid; CM= codlemone; DA= pear ester; T4 and T5 are coded due to commercial-in-confidence.

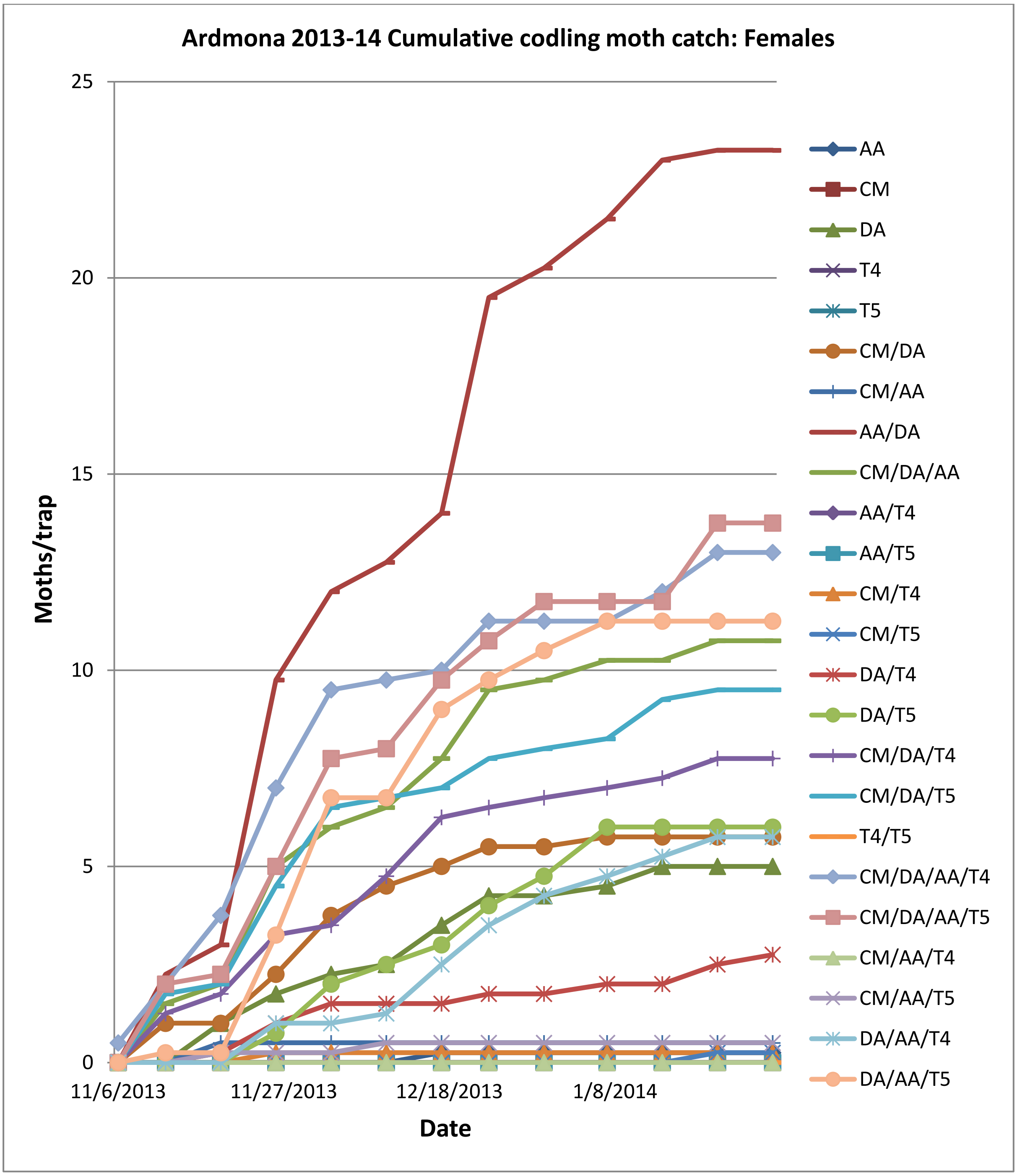

Table 2. ReML estimates of mean log(females +1) for Ardmona. Numbers followed by the same letter in the Mean column are not significantly different. Only the five best performing lures are annotated and these were significantly different to the unannotated lures. There were significant effects of Date, Lure, and Lure x Date interaction. F Prob (Date) < 0.001; F Prob (Lure) < 0.001; F Prob (Lure x Date) < 0.001. SEd(Date) = 0.02104; SEd(Lure) =0.02871; SEd (Lure x Date) =0.1108. AA= acetic acid; CM= codlemone; DA= pear ester; T4 and T5 are coded due to commercial-in-confidence.

Lure	6-Nov-13	12-Nov-13	19-Nov-13	26-Nov-13	3-Dec-13	10-Dec-13	17-Dec-13	23-Dec-13	30-Dec-13	7-Jan-14	14-Jan-14	21-Jan-14	28-Jan-14	5-Feb-14	12-Feb-14	Mean
AA	0.000	0.000	0.000	0.000	0.000	0.000	0.075	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.005
AA/DA	0.000	0.420	0.195	0.868	0.420	0.195	0.250	0.770	0.195	0.270	0.325	0.075	0.000	0.000	0.314	0.286[a]
AA/T5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
AA/T4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CM	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CM/AA	0.000	0.000	0.151	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010
CM/AA/T5	0.000	0.000	0.075	0.000	0.000	0.075	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010
CM/AA/T4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CM/DA	0.000	0.226	0.000	0.301	0.211	0.195	0.119	0.151	0.000	0.075	0.000	0.000	0.000	0.000	0.000	0.085
CM/DA/AA	0.000	0.345	0.119	0.508	0.239	0.151	0.250	0.369	0.075	0.151	0.000	0.151	0.000	0.151	0.433	0.196[b]
CM/DA/AA/T5	0.000	0.331	0.075	0.433	0.406	0.075	0.369	0.239	0.226	0.000	0.000	0.401	0.000	0.075	0.389	0.201[b]
CM/DA/AA/T4	0.119	0.301	0.389	0.508	0.476	0.075	0.075	0.301	0.000	0.000	0.195	0.226	0.000	0.000	0.420	0.205[b]
CM/DA/T5	0.000	0.376	0.075	0.464	0.331	0.075	0.075	0.195	0.075	0.075	0.175	0.075	0.000	0.000	0.075	0.138
CM/DA/T4	0.000	0.314	0.119	0.211	0.075	0.250	0.358	0.075	0.075	0.075	0.075	0.119	0.000	0.000	0.000	0.117
CM/T5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.075	0.000	0.000	0.000	0.005
CM/T4	0.000	0.000	0.000	0.075	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005
DA	0.000	0.000	0.226	0.226	0.119	0.075	0.226	0.151	0.000	0.075	0.119	0.000	0.000	0.000	0.000	0.081
DA/AA/T5	0.000	0.075	0.000	0.575	0.644	0.000	0.489	0.226	0.195	0.226	0.000	0.000	0.000	0.075	0.520	0.201[b]
DA/AA/T4	0.000	0.000	0.000	0.175	0.000	0.075	0.270	0.270	0.226	0.151	0.119	0.151	0.000	0.000	0.226	0.111
DA/T5	0.000	0.000	0.000	0.195	0.195	0.119	0.151	0.239	0.195	0.270	0.000	0.000	0.000	0.000	0.000	0.091
DA/T4	0.000	0.000	0.075	0.195	0.151	0.000	0.000	0.075	0.000	0.075	0.000	0.119	0.075	0.000	0.119	0.059
T5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
T4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
T4/T5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mean	0.005	0.100	0.062	0.197	0.136	0.057	0.113	0.127	0.053	0.060	0.042	0.058	0.003	0.013	0.104	

Figure 3: Cumulative catch of codling moth (both sexes combined) averaged over 4 replications for each treatment (combination of volatiles) at Bunbartha. AA= acetic acid; CM= codlemone; DA= pear ester; T4 and T5 are coded commercial-in-confidence.

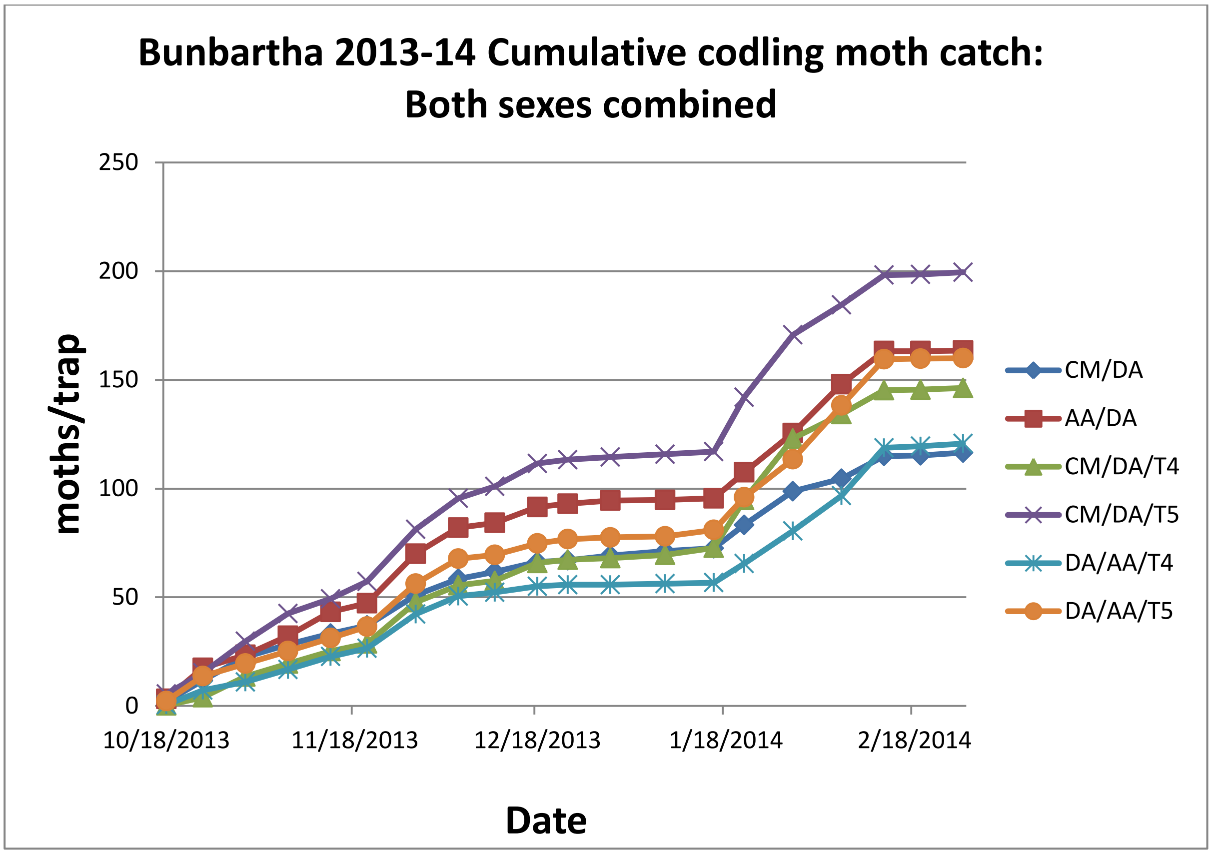

Table 3. ReML estimates of mean log(total catch + 1) for Bunbartha. Numbers followed by the same letter in the Mean row are not significantly different. There were significant effects of Date, Lure, and Lure x Date interaction. F Prob (Date) < 0.001; F Prob (Lure) < 0.001; F Prob (Lure x Date) < 0.001. SEd(Date) = 0.05985; SEd(Lure) = 0.03698 ; SEd (Lure x Date) = 0.1466. AA= acetic acid; CM= codlemone; DA= pear ester; T4 and T5 are coded due to commercial-in-confidence.

Lure	AA/DA	CM/DA	CM/DA/T5	CM/DA/T4	DA/AA/T5	DA/AA/T4	Mean
18-Oct-13	0.496	0.464	0.799	0.075	0.433	0.151	0.403
24-Oct-13	1.158	1.001	1.019	0.656	1.040	0.834	0.951
31-Oct-13	0.809	1.055	1.165	0.929	0.828	0.571	0.893
7-Nov-13	0.978	0.783	1.138	0.809	0.823	0.785	0.886
14-Nov-13	1.000	0.763	0.883	0.801	0.834	0.826	0.851
20-Nov-13	0.639	0.626	0.934	0.520	0.758	0.571	0.675
28-Nov-13	1.370	1.174	1.363	1.291	1.310	1.170	1.280
5-Dec-13	1.081	0.867	1.135	0.924	1.085	0.926	1.003
11-Dec-13	0.496	0.498	0.770	0.420	0.420	0.389	0.499
18-Dec-13	0.905	0.731	0.995	0.960	0.734	0.564	0.815
23-Dec-13	0.345	0.226	0.369	0.294	0.420	0.195	0.308
30-Dec-13	0.389	0.406	0.314	0.195	0.226	0.000	0.255
8-Jan-14	0.075	0.452	0.250	0.325	0.151	0.119	0.229
16-Jan-14	0.195	0.314	0.301	0.615	0.527	0.151	0.350
21-Jan-14	1.097	1.048	1.403	1.320	1.156	0.967	1.165
29-Jan-14	1.227	1.201	1.458	1.418	1.200	1.190	1.282
6-Feb-14	1.370	0.822	1.143	1.037	1.378	1.235	1.164
13-Feb-14	1.167	1.015	1.117	1.004	1.277	1.336	1.153
19-Feb-14	0.000	0.075	0.075	0.075	0.075	0.226	0.088
26-Feb-14	0.075	0.345	0.226	0.195	0.075	0.301	0.203
Mean	0.7436^{b}	0.6932^{bc}	0.8428^{a}	0.6931^{bc}	0.7375^{b}	0.6253^{c}	

Figure 4: Cumulative catch of codling moth females averaged over 4 replications for each treatment (combination of volatiles) at Bunbartha. AA= acetic acid; CM= codlemone; DA= pear ester; T4 and T5 are coded commercial-in-confidence.

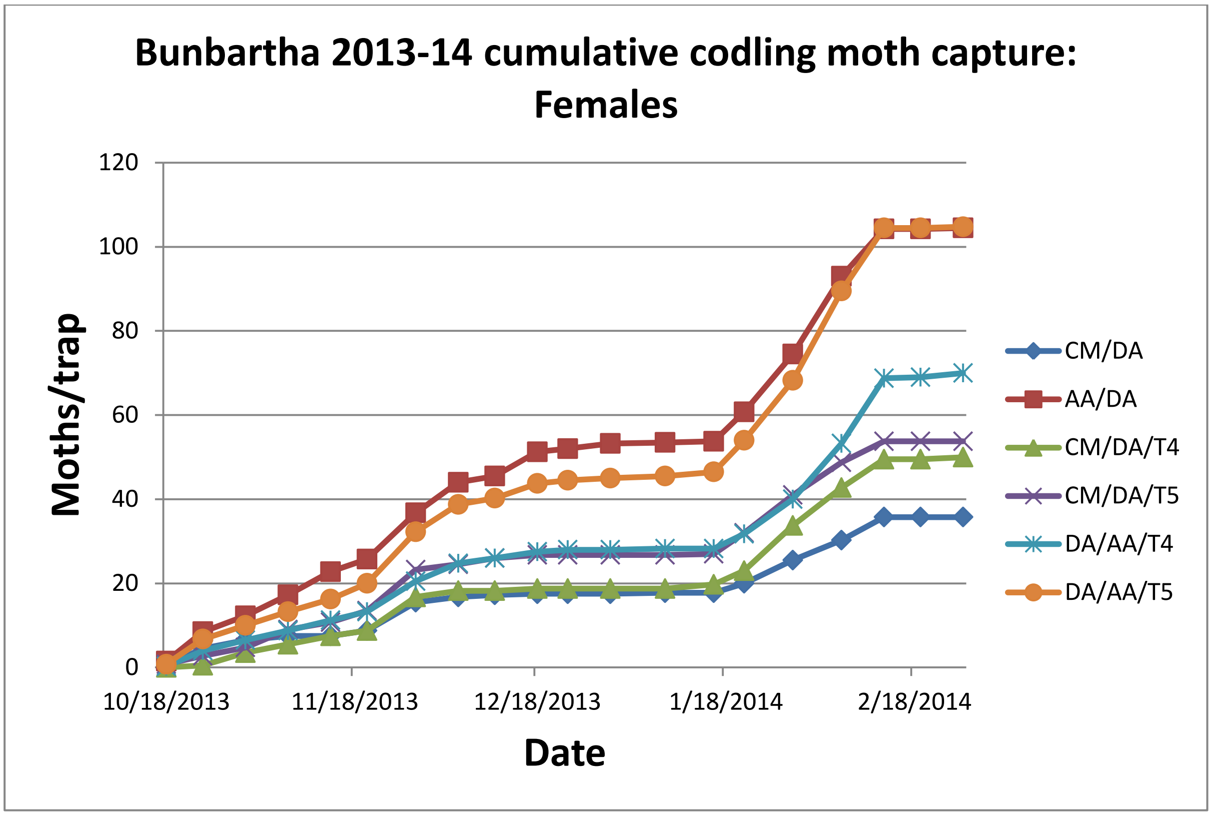

Table 4. ReML estimates of mean log(females + 1) for Bunbartha. Numbers followed by the same letter in the Mean row are not significantly different. There were significant effects of Date, Lure, and Lure x Date interaction. F Prob (Date) < 0.001; F Prob (Lure) < 0.001; F Prob (Lure x Date) = 0.001. SEd(Date) = 0.05863; SEd(Lure) = 0.03484; SEd (Lure x Date) = 0.1436. AA= acetic acid; CM= codlemone; DA= pear ester; T4 and T5 are coded due to commercial-in-confidence.

Lure	AA/DA	CM/DA	CM/DA/T5	CM/DA/T4	DA/AA/T5	DA/AA/T4	Mean
18-Oct-13	0.345	0.119	0.151	0.000	0.195	0.075	0.147
24-Oct-13	0.876	0.687	0.350	0.151	0.821	0.663	0.591
31-Oct-13	0.663	0.420	0.401	0.588	0.626	0.464	0.527
7-Nov-13	0.765	0.226	0.683	0.420	0.619	0.489	0.534
14-Nov-13	0.644	0.000	0.314	0.464	0.595	0.496	0.419
20-Nov-13	0.569	0.314	0.489	0.270	0.656	0.401	0.450
28-Nov-13	1.054	0.887	0.992	0.882	1.112	0.886	0.969
5-Dec-13	0.869	0.270	0.270	0.301	0.863	0.707	0.547
11-Dec-13	0.376	0.119	0.345	0.000	0.345	0.314	0.250
18-Dec-13	0.811	0.075	0.195	0.119	0.600	0.389	0.365
23-Dec-13	0.195	0.000	0.000	0.000	0.195	0.151	0.090
30-Dec-13	0.345	0.000	0.000	0.000	0.151	0.000	0.083
8-Jan-14	0.075	0.075	0.000	0.000	0.151	0.075	0.063
16-Jan-14	0.075	0.000	0.075	0.270	0.270	0.000	0.115
21-Jan-14	0.848	0.362	0.750	0.537	0.926	0.584	0.668
29-Jan-14	1.098	0.795	0.977	1.012	1.088	0.960	0.988
6-Feb-14	1.284	0.755	0.926	0.951	1.322	1.152	1.065
13-Feb-14	1.051	0.775	0.710	0.790	1.153	1.175	0.942
19-Feb-14	0.000	0.000	0.000	0.000	0.000	0.075	0.013
26-Feb-14	0.075	0.000	0.000	0.151	0.075	0.270	0.095
Mean	0.601^{a}	0.294^{c}	0.381^{c}	0.345^{c}	0.588^{a}	0.466^{b}	

Discussion

Lures containing codlemone alone only attract male codling moths. Addition of pear ester (DA) to codlemone (CM)-baited traps in orchards under mating disruption enhances capture of males and also attracts females. Recent research suggests that addition of acetic acid to traps baited with CM/DA further increases capture of moths but this is not supported by our results for both sexes combined. The seven best performing lures based on total catch (both sexes combined) at Ardmona were, in descending order CM/DA/T5, CM/DA, CM/DA/AA/T4, CM/DA/AA, CM/DA/AA/T5, CM/DA/T4, and AA/DA (Fig. 1) although the numbers caught in traps baited with these lures were not significantly different (Table 1). Traps baited with CM/DA/T5 caught significantly more moths (both sexes combined) than the other lures, including CM/DA, at Bunbartha (Fig. 3, Table 3). This may indicate a varietal influence between Packham and Corella pear volatiles.

The best performing lure for capturing female codling moths at Ardmona was AA/DA (Fig.2, Table 2) with AA/DA significantly better than the next best performers CM/DA/AA/T4, CM/DA/AA/T5, CM/DA/AA, DA/AA/T5, and CM/DA/AA (none of which were significantly better than each other). In the Bunbartha experiment there were no significant differences between the two best performers AA/DA and DA/AA/T5 but they were both significantly different to DA/AA/T4 which in turn was significantly better than CM/DA/T5, CM/DA/T4, and CM/DA (Fig 4, Table 4).

Further work is required to understand the influence of the individual components within the mixtures, and to explore possible influence of pear varietal differences in volatile emissions. The data collected in the 2013-14 season indicate that CM/DA/T5 could be a useful combination for mass-trapping both sexes of codling moth in orchards under pheromone-mediated mating disruption.

Appendix 2:

Active space and required number of traps baited with a combination of semiochemicals for mass-trapping of codling moth in pear orchards

D.G. Williams[1], A.L.Il'Ichev[1], and S.Chandra[2]

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Agriculture Victoria
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Introduction

In studies on volatile attractants, the active space is the area around a source point in which the semiochemical concentration is above a behavioural threshold eliciting positive orientation by the moth. The number of traps/ha required to reliably monitor a moth population in an orchard depends on the active space of the trap, the behaviour of the target moth species, and spatial distribution of the moth population in the orchard. Traps using combinations of semiochemicals could be useful tools for both monitoring and/or mass-trapping pest insects, especially in orchards where pheromone-based mating disruption (MD) is being used as a pest management tool and inadvertently decreases the efficacy of normal pheromone traps as monitoring tools.

The aims of the work reported here are to determine the spatial density of traps required to reliably monitor the moth population, confirm the active space of traps in orchards under MD, and investigate sex-related differences to attraction of moths to traps. The information generated by this research will then be applied in 2015-16 field experiments investigating the efficacy of the traps as mass-trapping devices.

Materials and Methods

A block of pears (cv Packham's Triumph) within an orchard at Ardmona, Victoria, Australia, was selected as the study site. The block had a history of damage by codling moth, and treatment with pheromone mediated mating disruption. Tree rows were 5.9 m apart and trees within the rows were spaced approximately 5.1 m apart. The experimental plot was approximately 2.6 ha in size. One delta trap baited with the test lure (codlemone + pear ester + a confidential host-plant volatile (T5)) was placed on a branch within the top meter in the outer edge of the canopy in every 2nd tree (starting 3 trees in from the western end of the row) in every 2nd row (starting 3 rows in from the northern side of the block) until 14 traps had been placed in each of 8 trapped rows. This resulted in a grid of 112 traps in which each trap was approximately 11.8 m away from its nearest neighbour across a row or 10.2 m within rows.

Another block of the same pear variety was used to compare active space of the traps when mating disruption pheromone was not present. This block was located about 145 m to the south of the first block, and had a similar history of codling moth damage, with trees the same age, and laid out the same way as in the first block. Traps were established using the same protocol as per the first block.

The location of all traps was recorded as GPS coordinates. Lures in the traps were not periodically changed (normal practice would be to change lures every 6-8 weeks) because we were interested in testing longevity of the lures as well as attractive radius. Traps were inspected each week for presence of moths. All trapped codling moths were counted and then collected into vials for transport to the laboratory to determine gender and mating status. Gender identification was performed by examining genitalia under the dissecting microscope. For identification of female mating status, the abdomen of CM females was dissected and bursa copulatrix inspected for presence of spermatophores.

The cumulative catch in each trap and the GPS referenced location of each trap, was used in Vesper 1.6 (Australian Centre for Precision Agriculture, The University of Sydney, NSW, Australia) to produce a spatial prediction of the codling moth population density in the orchard at each sampling date. This then provided guidance as to appropriate transformation of data for calculating active radius.

To determine a sample size (number of traps) that adequately represented the average catch in the population of 112 traps, we generated 200 random samples of each of 2-15, 20, 25, 30, 35, 40, 45, and 50 traps using simple random sampling without replacement. The arithmetic mean of each of these 200 random samples was then computed. The distribution of these 200 mean values for different sample sizes was summarized using box plots for female, male and total catch. Box plots were also produced for the distribution of Coefficient of Variation (CV) from the same data.

To explore the extent of spatial dependence between traps we fitted variograms to the spatial data for moth catches using models with 200 lags, lag tolerance= 0.5, maximum distance = 100m, and weighting= number of pairs. The point of inflexion, or the start of a sill, in the variograms indicates the distance beyond which capture of moths in traps cease to be spatially dependent. There were high numbers of zero catches throughout the season due to spatial variation in population density, so the data were log(x+1) transformed before fitting a variogram using a linear-with-sill model in Vesper 1.6. The active space of a trap that relies on a plume of volatiles for attraction is represented in two dimensions as the area of a circle centred on the trap. Any overlap of the active spaces indicates competition between the traps. The point at which two traps cease interfering with each other is the point at which the two circumferences meet but not overlap. The length of the line, drawn between the traps, that passes through this point is the separation distance indicated by the variogram. If the two traps are identical then the length of the line between the two traps is twice the radius of the active space.

Results and Discussion

Figure 1 displays the results from the trapping, presented as cumulative number of moths captured over the 2014-15 season. The results show the rate of capture of female moths in both MD and non-MD treated blocks decreased after 3 weeks of trapping whereas the rate of male capture was relatively consistent throughout the season. More females than males were caught in the MD treated block in the 2nd and 3rd weeks (Figure 2).This suggests that either the female population was rapidly depleted in the first 3-4 weeks or the new female attractant component of the lure was depleted after 3 weeks, and females continued to be captured throughout the rest of the season as a result of continued emission of the pear ester component of the lure. A similar result was observed in the non-MD treated block (Figure 3) although in that case the number of females caught never exceeded the number of males. Damage levels to the crop by codling moth were 0.57% for the MD treated block and 0.78% in the non-MD treated block. Such low levels of damage suggest that the mass trapping may have significantly reduced moth populations. If the lure components had depleted then more frequent replacement of the lures, or a change of formulation to ensure better longevity, should enhance female capture.

Figure 1: 2014-2015 codling moth mass-trapping trial. Cumulative numbers of male, female and both sexes combined are displayed, captured in Packham pear blocks treated with (MD+) or without (MD-) pherome-based mating disruption.

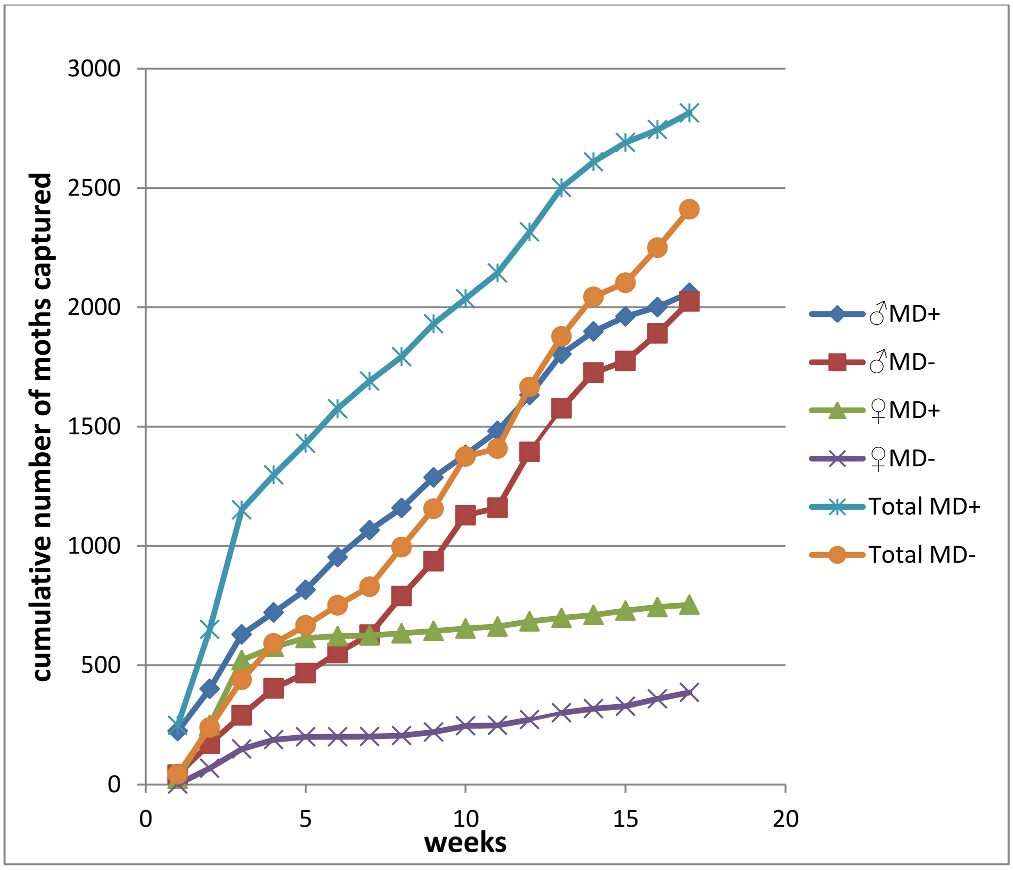


Figure 2: Total number of moths captured each week in the MD+ block

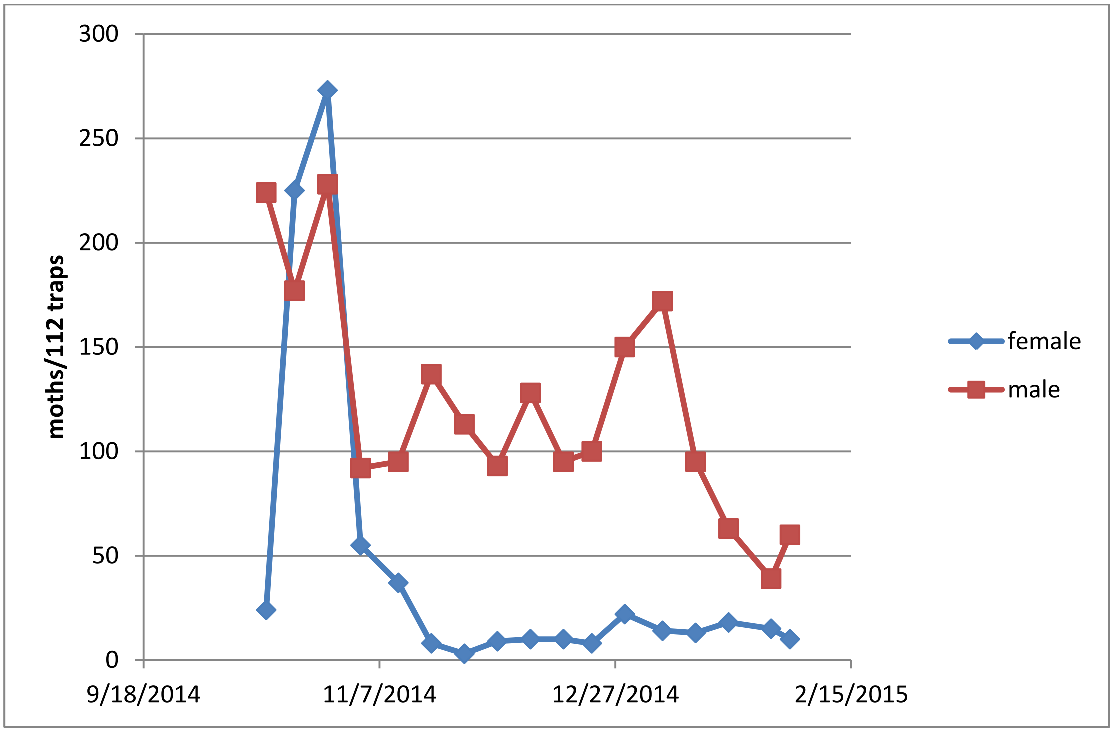
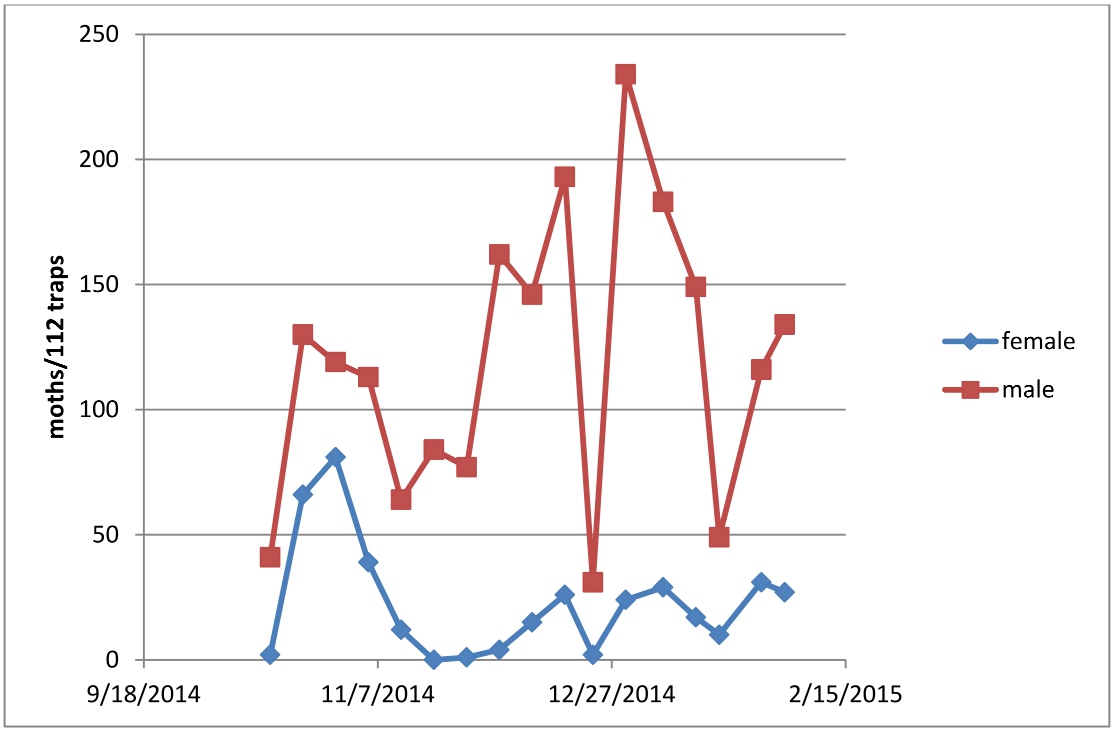


Figure 3: Total number of moths captured in the non-MD block



The variograms for the plot under mating disruption were influenced by relatively low population levels during the mid to late part of the season. Variograms for the early part of the season when the populations were high demonstrated a sill at 67 m for females (Figure 4) and 86 m for males (Figure 5), which translate to active radius of 33.5 m and 43 m respectively. The variograms for the control plot (not under mating disruption) did not yield a relationship for female moths but the active radius for male moths averaged 29.5 m.

Figure 4: Variogram for capture of female codling moths in MD treated pears.

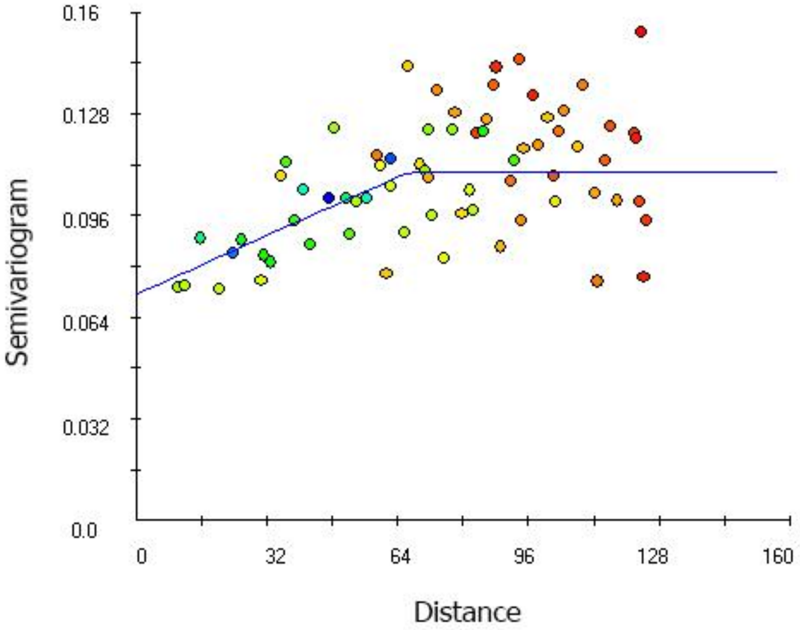
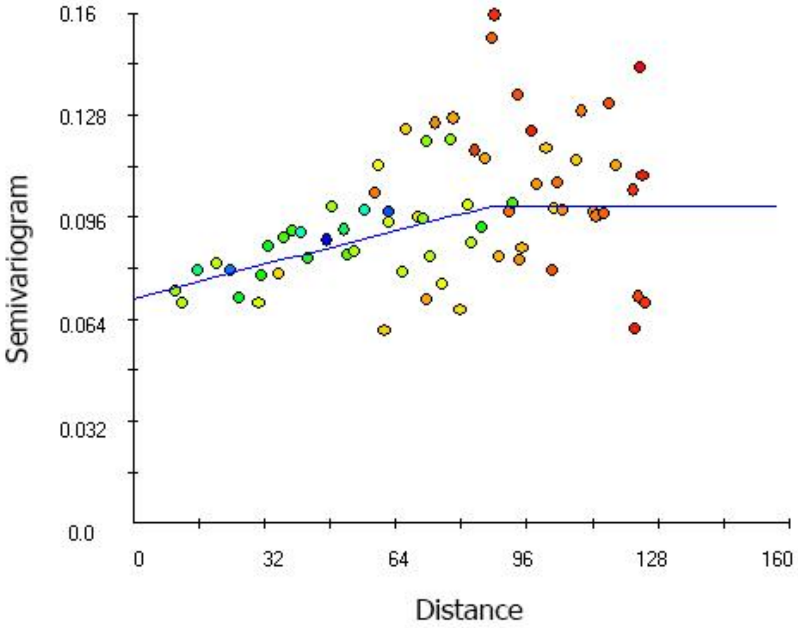


Figure 5: Variogram for capture of male codling moths in MD treated pears.



Inspection of the box plots for mean values of population estimates from various numbers of traps indicated that at least 20 traps were required to obtain consistent results (Figures 6-8). Scaling down from the 2.6 ha plot to one hectare yields 8 traps/ha as a minimum in blocks treated with mating disruption. At that trapping density each trap would service 10000 m^2/8 = 1250 m^2 and therefore have an active radius of approximately 20 m.

Figure 6: Example of box plot output mean population of (male and female combined) codling moths in a mating disruption treated block vs number of traps sampled.

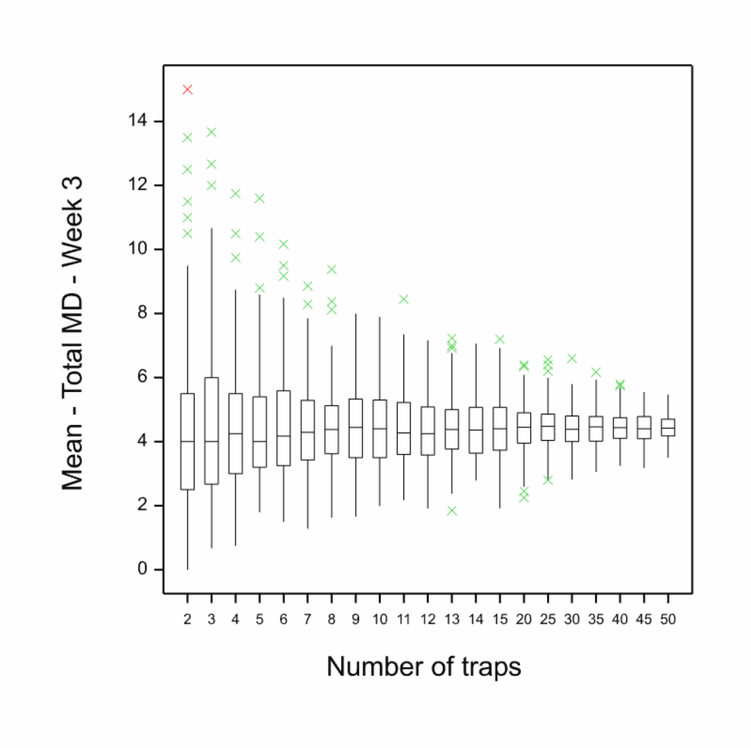


Figure 7: Example of box plot output mean population of male codling moths in a mating disruption treated block vs number of traps sampled.

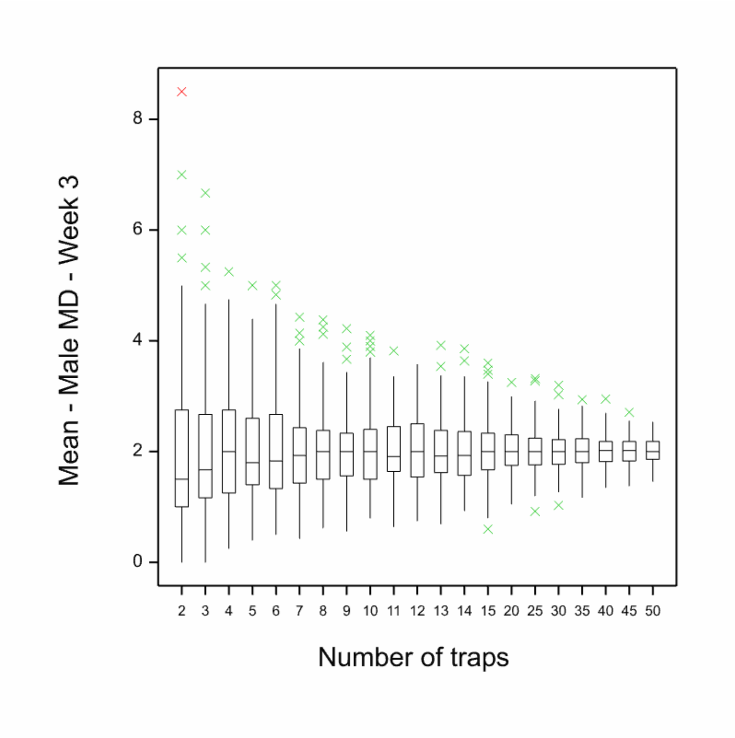
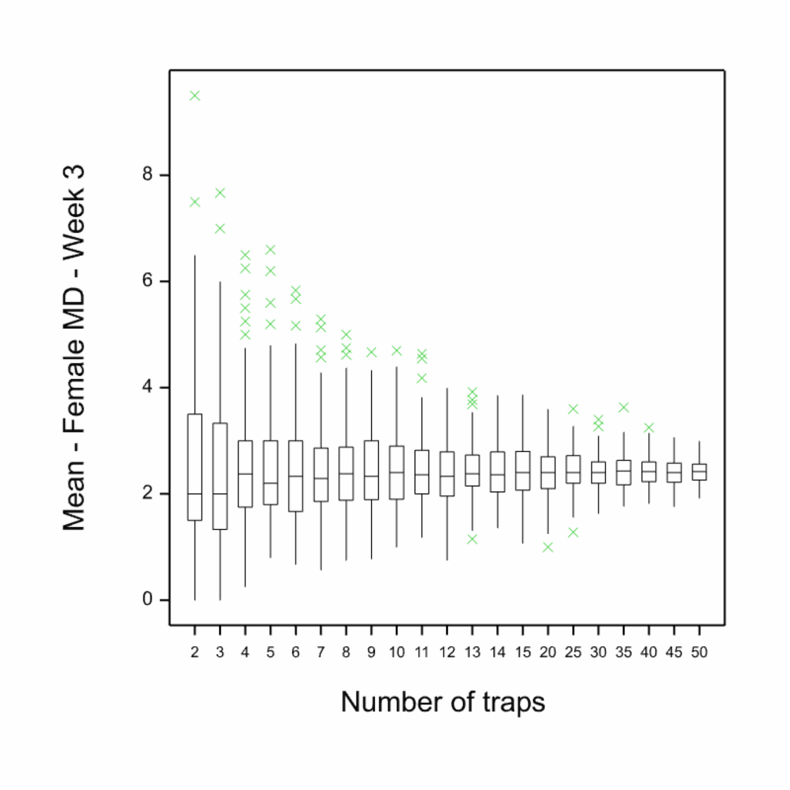


Figure 8: Example of box plot output mean population of female codling moths in a mating disruption treated block vs number of traps sampled.



The 2014-15 results identified two important questions that require attention:

- The need to explore whether the decline in capture of female moths was due to depletion of the female population (which could explain the low level of damage to fruit recorded at harvest) or depletion of the female attractant in the lure. This was explored in the 2015-16 work plan (Appendix 3)
- The calculation of active radius as 33-43m equates to 2-3 traps/ha and appears at odds with the simulation study that suggests that 20 traps/ 2.6 ha (or 8 traps/ha) were required to accurately determine the spatial density of the codling moth population. This translates roughly to an active radius of 20m. The apparent discrepancy between the two calculations of active radius may be due to the variogram-based method using the radius of circles whose circumferences just touch but do not overlap. It is not designed to test the number of traps required to determine population levels, and results in gaps between active spaces when traps are deployed at separation distances equal to, or greater than, the diameter of their active space. The simulation study was designed to calculate the number of traps required to accurately determine spatial density of the moth population and allowed for overlap of active spaces that probably accounted for the aggregated nature of the codling moth infestations within the blocks. The next steps (2015-16) are to demonstrate the efficacy of a mass-trapping system to control codling moth. Once efficacy has been demonstrated further work will be required to reconcile the issues raised above and fine tune the trap design to make the system cost-effective.

Appendix 3:

Mass-trapping of codling moth in orchards

D.G. Williams[1], A.L.Il'Ichev[1], and S.Chandra[2]

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Introduction

Volatile attractants create an active space around a source point. The active space is the area in which the semiochemical concentration is above a behavioural threshold eliciting orientation by the moth towards a source point. The number of traps/ha required to reliably monitor a moth population in an orchard depends on the active space of the trap, the behaviour of the target moth species, and spatial distribution of the moth population in the orchard. Traps using combinations of semiochemicals could be useful tools for both monitoring and/or mass-trapping pest insects, especially in orchards where pheromone-based mating disruption (MD) is being used as a pest management tool and inadvertently decreases the efficacy of normal pheromone traps as monitoring tools. Previous work in this project has identified a useful mix of pheromone and host-plant volatiles for use in trapping both male and female codling moths in orchards. Results from last season suggested that the female attractant component of the lure may have been depleted after 3 weeks field exposure.

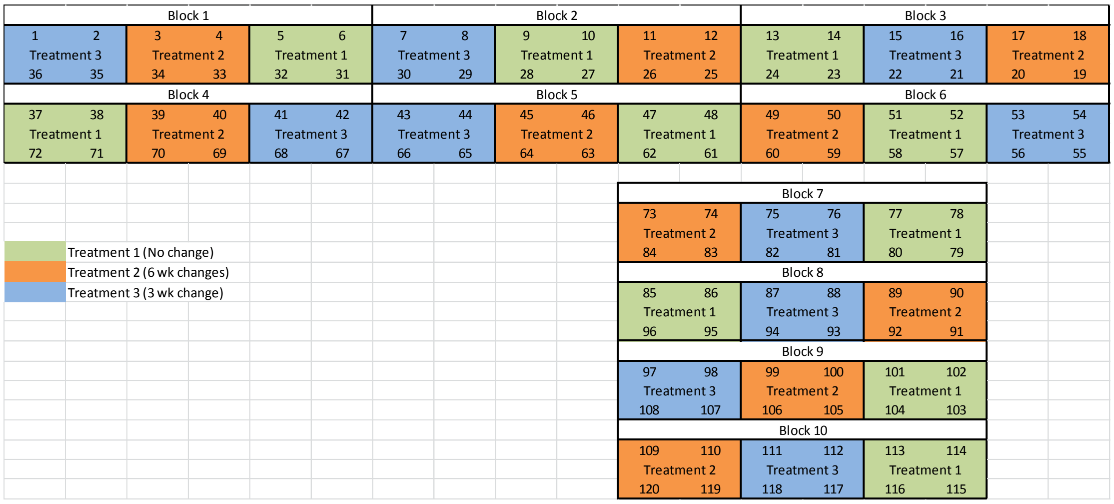
The aims of the work reported here are to assess the field life of the lure, determine potential impacts on efficacy of mass-trapping, and to confirm the radius of the trap active space.

Materials and Methods

The same pear blocks at Ardmona, used in previous seasons, were selected as the study site. One delta trap baited with the test lure (codlemone + pear ester + a confidential host-plant volatile (T5)) was placed on a branch within the top meter in the outer edge of the canopy of each of 4 trees per plot, with 3 plots (treatments) per experimental block, and 10 blocks in total. Treatments allocated to plots were based on frequency of changing the lures. Treatments were (1) no change of lure; (2) lure changed every 6 weeks; and (3) lure changed every 3 weeks. Layout is shown in Figure 1.

The location of all traps was recorded as GPS coordinates so that spatial analysis could be used to confirm the active radius calculations from the previous season. Traps were inspected each week for presence of moths. All trapped codling moths were counted and then collected into vials for transport to the laboratory to determine gender by examining genitalia under the dissecting microscope.

Figure 1: Plot, Block and Treatment allocation.



Block 1			Block 2			Block 3		
1 2 Treatment 3 36 35	3 4 Treatment 2 34 33	5 6 Treatment 1 32 31	7 8 Treatment 3 30 29	9 10 Treatment 1 28 27	11 12 Treatment 2 26 25	13 14 Treatment 1 24 23	15 16 Treatment 3 22 21	17 18 Treatment 2 20 19
Block 4			**Block 5**			**Block 6**		
37 38 Treatment 1 72 71	39 40 Treatment 2 70 69	41 42 Treatment 3 68 67	43 44 Treatment 3 66 65	45 46 Treatment 2 64 63	47 48 Treatment 1 62 61	49 50 Treatment 2 60 59	51 52 Treatment 1 58 57	53 54 Treatment 3 56 55

Block 7		
73 74 Treatment 2 84 83	75 76 Treatment 3 82 81	77 78 Treatment 1 80 79
Block 8		
85 86 Treatment 1 96 95	87 88 Treatment 3 94 93	89 90 Treatment 2 92 91
Block 9		
97 98 Treatment 3 108 107	99 100 Treatment 2 106 105	101 102 Treatment 1 104 103
Block 10		
109 110 Treatment 2 120 119	111 112 Treatment 3 118 117	113 114 Treatment 1 116 115

🟩 Treatment 1 (No change)
🟧 Treatment 2 (6 wk changes)
🟦 Treatment 3 (3 wk change)

Results and Discussion

There were no statistically significant differences between treatments (F Prob: 0.620 males, 0.331 females) or treatment X week interactions (F Prob: 0.079 males, 0.060 females) but there was a significant effect of week (F Prob: <0.001 in both cases) in terms of cumulative numbers of moths captured in traps that did not have the lures changed for the season (treatment 1), had lures changed every 6 weeks (treatment 2), or had lures changed every 3 weeks (treatment 3) (Table 1). However, in the last four weeks of trapping comparison of the means using LSD 5% indicated that treatment 2 caught significantly more female moths than treatment 1, whereas the results for treatment 2 vs treatment 3, and treatment 3 vs treatment 1 were not significantly different (Figure 2). This is difficult to explain in terms of lure longevity since treatment 3 had the most frequent changes of lure and was expected to perform better than the other two treatments if longevity was the issue, as suspected at the end of 2014-15. The situation was reversed with capture of male moths, with treatment 3 capturing significantly more moths than treatment 1, and captures in treatment 2 not being significantly different to those in treatment 1 or treatment 3 (Figure 3). The results suggest that the lures last for at least 6 weeks.

Variograms fitted to total male capture (Figure 4), total female capture (Figure 5), and total moths (both sexes combined)(Figure 6) indicated separation distances of 69 m, 63 m, and 59 m respectively, which translate to active radius of 34.5 m, 31.5 m, and 29.5 m for males, females and total respectively. These confirm the results of the 2014-15 trials.

Fruit damage was 0.083% at harvest. This is in line with the forecast outcome of less than 0.1% crop damage, which was provided in the project submission.

Table 1. Comparison of mean cumulative number of codling moths captured in traps baited with lures changed at three different frequencies (T1 = no change; T2 = change every 6 weeks; T3 = change every 3 weeks)

Mean cumulative number of males					**Mean cumulative number of females**				
	Treat					Treat			
Week	T1	T2	T3	Mean	Week	T1	T2	T3	Mean
1	6.1	6.8	6.5	6.4	1	6.5	8.0	5.4	6.6
2	6.7	7.6	7.1	7.1	2	6.7	8.5	5.8	7.0
3	7.9	8.9	8.2	8.3	3	7.0	8.7	6.0	7.2
4	9.7	10.2	9.7	9.9	4	7.6	9.2	6.6	7.8
5	11.0	11.1	10.7	10.9	5	7.8	9.4	6.7	8.0
6	12.3	12.6	12.6	12.5	6	8.3	9.9	7.5	8.5
7	13.0	13.2	13.3	13.2	7	8.5	10.1	7.7	8.7
8	13.9	14.4	14.5	14.2	8	8.7	10.3	7.8	8.9
9	15.8	16.6	16.6	16.3	9	9.6	11.4	8.7	9.9
10	16.5	17.7	17.9	17.4	10	9.9	11.8	9.3	10.3
11	17.3	18.8	18.9	18.3	11	10.3	12.5	9.8	10.9
12	17.8	19.3	20.0	19.0	12	10.4	12.7	10.0	11.0
13	18.1	20.1	20.5	19.6	13	10.5	13.0	10.2	11.2
14	18.5	20.6	21.2	20.1	14	10.8	13.3	11.0	11.7
15	19.0	21.4	22.2	20.8	15	10.8	13.8	11.7	12.1
16	19.4	21.8	22.7	21.3	16	10.9	14.0	11.9	12.3
17	19.4	22.0	22.8	21.4	17	10.9	14.0	12.0	12.3
18	19.5	22.2	23.2	21.6	18	10.9	14.0	12.1	12.3
Mean	14.5	15.8	16.0		Mean	9.2	11.4	8.9	

males:

F Prob: Treat *0.620*, Week *<0.001*, Treat x Week *0.079*

LSD 5%: Treat *3.44*, Week *0.82*, Treat x Week *3.96*

females:

F Prob: Treat *0.331*, Week *<0.001*, Treat x Week *0.060*

LSD 5%: Treat *3.67*, Week *0.51*, Treat x Week *4.17*

Figure 2. Seasonal (2015-16) cumulative capture of female moths in traps baited with lures that were not changed for the duration (T1), changed every 6 weeks (T2), or changed every 3 weeks (T3). The vertical bar at the left side represents LSD 5%.

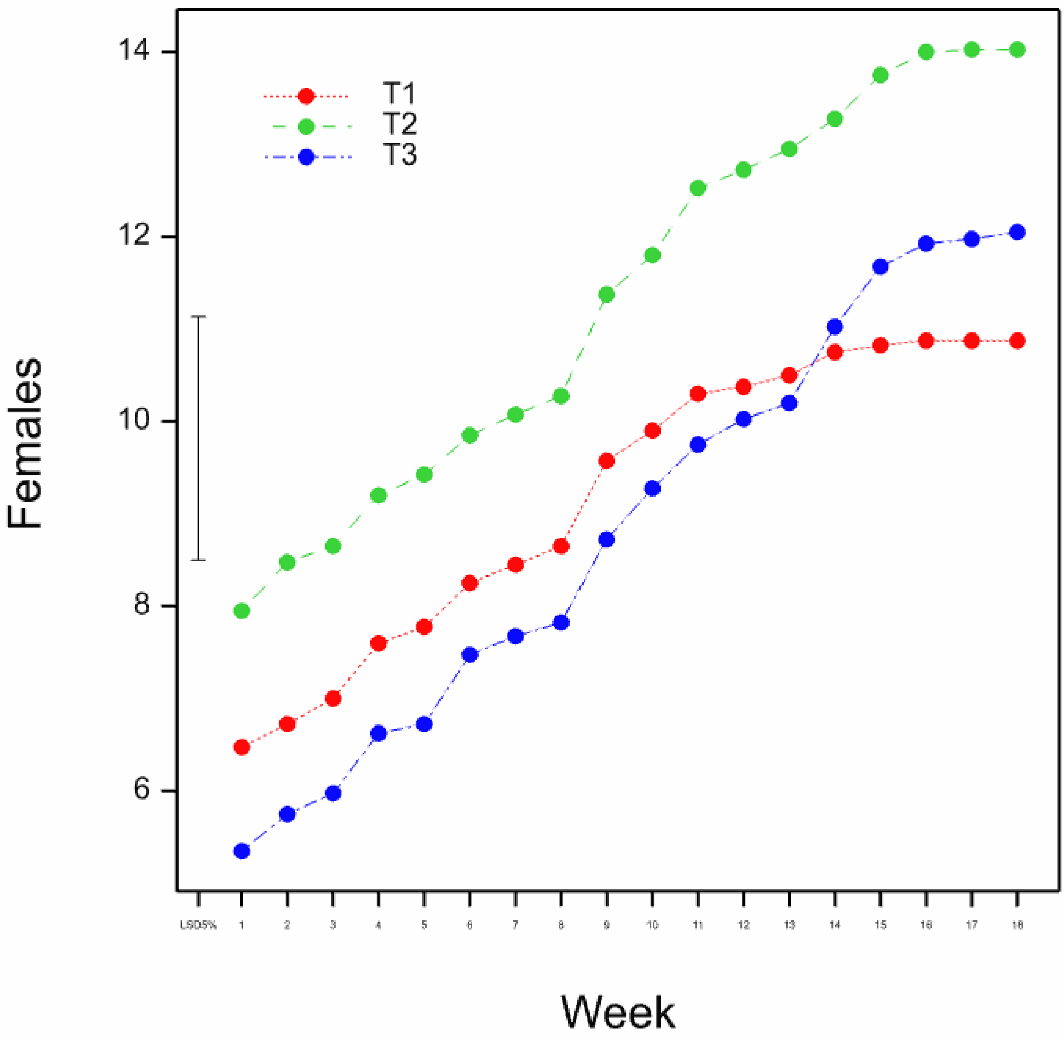


Figure 3. Seasonal(2015-16) cumulative capture of male moths in traps baited with lures that were not changed for the duration (T1), changed every 6 weeks (T2), or changed every 3 weeks. The vertical bar at the left side represents LSD 5%.

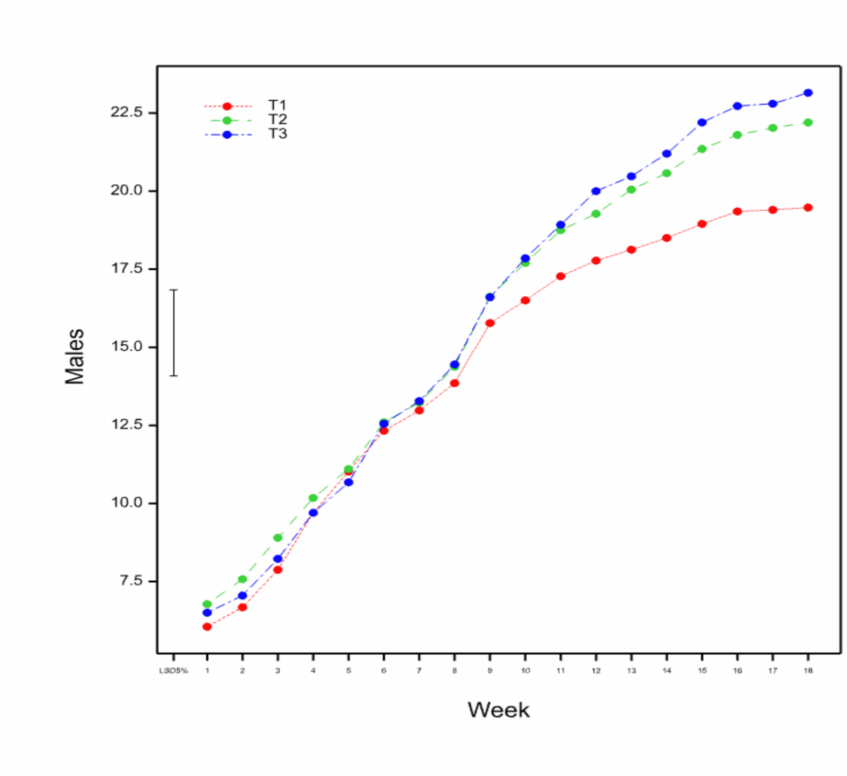


Figure 4: Variogram (30 lags, 50% tolerance, max distance 100) fitted to numbers of male moths captured in traps 2015-16. Sill point occurs at 69m.

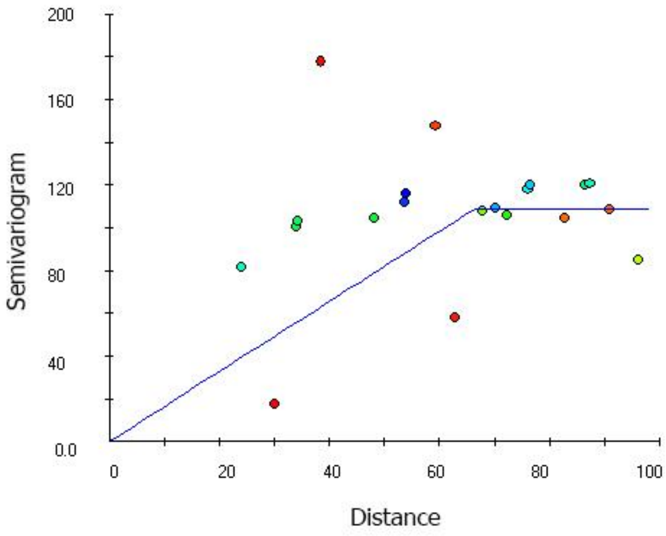


Figure 5: Variogram (30 lags, 50% tolerance, max distance 100) fitted to numbers of female moths captured in traps 2015-16. Sill point occurs at 63m.

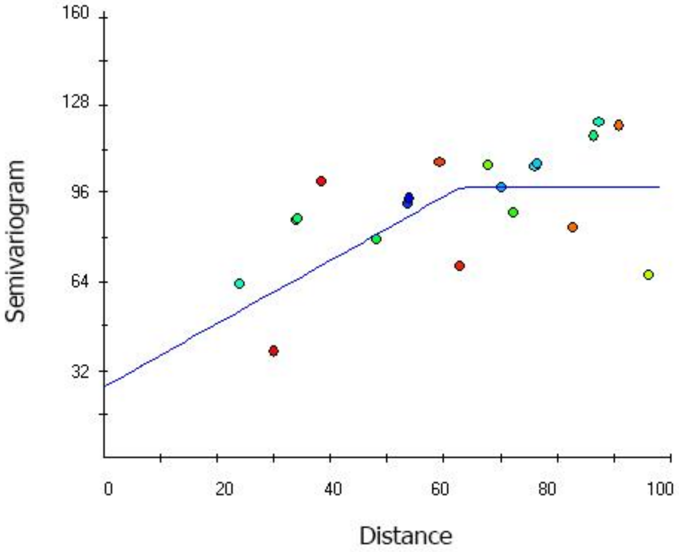
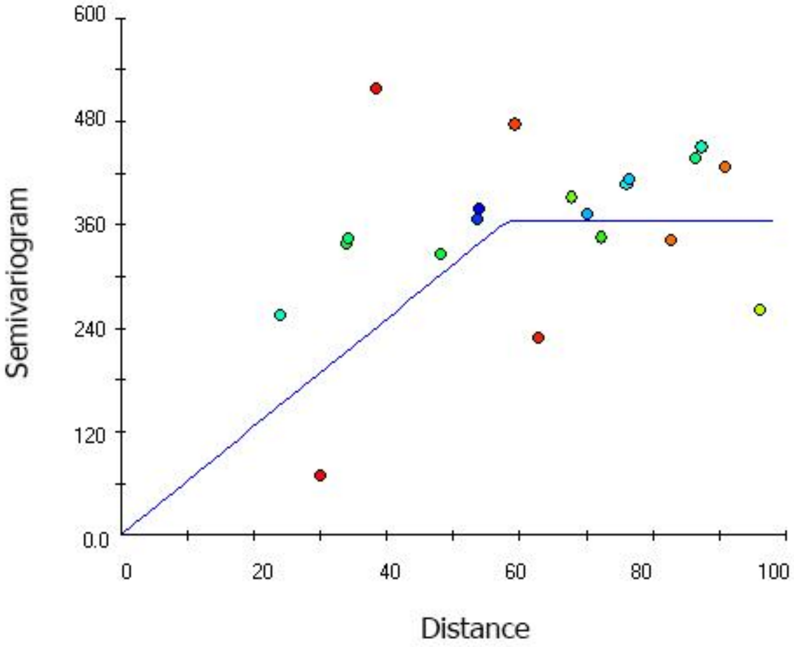


Figure 6: Variogram (30 lags, 50% tolerance, max distance 100) fitted to numbers of moths (both sexes combined) captured in traps 2015-16. Sill point occurs at 59m.



Appendix 4:

Integrating mass-trapping of codling moth into orchard pest management strategies

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Changes to the types of pesticide available for use in fruit production, and the progress of research into biological control of major insect pests, is providing fruit growers with safer, cost-effective, and environmentally friendly options to incorporate into their pest management systems.

Codling moth *Cydia pomonella* L. (CM) is a serious pest of pome fruit worldwide and the most damaging pest of commercial apple, pear, quince and nashi (Asian pears) orchards in Australia. Codling moth is widely distributed in Australian states except the Northern Territory and Western Australia, which is considered to be free of CM after a successful eradication program (Poole 2004). Uncontrolled CM moth larvae can destroy significant amounts of the crop (Geier 1963).

In the past CM was controlled by multiple applications of organophosphate insecticides (Thwaite et al. 1993) but these have been replaced by newer, narrower-spectrum pesticides that have lower human toxicity. Insect phenology models are an important tool in integrated pest management (IPM), allowing for more accurate timing of insecticides to sensitive periods in insect development. Predictive phenology models based on the timing and capture of male moths in the sex pheromone traps and temperature-dependent physiological development have been widely implemented to time insecticide applications (Glenn 1922, Hagley 1973, Croft and Knight 1983, Rice et al. 1984, Williams 1989, Knight and Light 2005, Knight 2007). These models can predict the start of egg hatch for each generation so that sprays aimed at newly hatched caterpillars can be better timed, and can also be modified to predict the best timing for application of sprays to kill eggs.

Application of sex pheromone mediated mating disruption is an effective alternative to the use of pesticides for control of low-moderate population levels of codling moth in Australian orchard IPM programs (Williams and Il'ichev 2003). Successful control of CM in pome fruit also has been achieved with the use of MD alone or in conjunction with limited insecticide treatments (Vickers and Rothschild 1991, Vickers et al. 1998). Although MD provides effective control of low-density CM populations, control of moderate-to-high population densities has been more problematic and often requires supplementary insecticide sprays (Vickers et al. 1998). A number of factors have been implicated in the poor performance of MD against high population densities. MD works by delaying mating through males experiencing sensory overload when they approach a MD dispenser. This has the effect of arresting male activity for a night each time they get close to a dispenser. However, if the female population density is high enough compared to the distribution density of the MD dispensers some males will find females instead of dispensers, and mating will occur.

Long-term successful control of CM with area-wide MD treatments of pome fruit was demonstrated in the western USA during 5 years of a government-supported program (Brunner et al. 2001). Incorporation of selective area-wide MD treatments of major pests into pheromone-based IPM programs has potential for development of cost effective strategies for controlling pests, while improving protection of the environment by reducing the amount of pesticides applied in orchards (Williams and Il'ichev 2003).

Recent research demonstrated that mature (5th instar) larvae of CM produce an aggregation pheromone that encourages larvae seeking overwintering and/or pupation sites to congregate close to each other on the tree. The aggregation pheromone only acts over relatively short distances and does not attract larvae from nearby trees (Jumean et al. 2004). In orchards with low CM population densities the larvae are sparsely distributed and are unlikely to detect the aggregation pheromone emanating from nearby sites on other trees. However, in orchards with high populations, or "hot-spots" within blocks, the aggregation pheromone would result in clustering of larvae. Males of CM develop faster than females and this means that adult males emerge earlier than females. Female pupae have recently been shown to emit sex pheromone prior to emergence of the adult female moth. The presence of sex pheromone in the clustered sites where the males are emerging arrests males who then are close enough to the emerging virgin female to be able to bypass the normal searching behaviour and once they visibly detect the female moth they exhibit courtship behaviour leading to copulation (Duthie et al. 2003). Pheromone mediated MD is not able to compete with such behaviour. It is therefore important to develop techniques that either reduce the population of overwintering codling moth larvae to levels controllable by MD, or to interfere with the ability of mated female codling moths to lay eggs.

Codling moth overwinters on pome fruit trees as diapausing (hibernating) mature caterpillars in cocoons in sheltered area such as under bark scales on the trunk. In spring as the daylength increases and temperatures warm up the hibernating caterpillars break diapause, enter pupation and eventually emerge as adult moths ready to mate and lay eggs. Mating disruption is designed to reduce or delay mating so that fewer eggs are laid. The parasitoid wasp *Mastrus ridens* seeks out hibernating codling moth caterpillars and lays eggs in the cocoon. The wasp eggs hatch and the larvae then feed on the codling moth caterpillars, killing them. The wasps have only recently been approved for release in Australia and it will be another few years before sufficient wasps have established to exert a major influence on codling moth populations.

In Australian stone fruit, OFM (oriental fruit moth, *Grapholita molesta* Busck) has been successfully controlled through the use of MD for more than 35 years (Rothschild 1975, Vickers 1990, Sexton and Il'ichev 2000). OFM also infests pome fruit and can be controlled in that crop by MD.

The woolly apple aphid (WAA) *Eriosoma lanigerum* (Hausmann) is a major pest of apple orchards worldwide (Blackman & Eastop 1994; Blommers 1994). WAA infestations can occur both below (on the root system) and above ground (on the stem and foliage) and can seriously reduce tree growth (Brown *et al*. 1995). Abundant populations on young trees can cause stunting or death (Blackman & Eastop 1994). Additionally, the production of honeydew acts as a source for sooty mould and when this establishes on fruit it can limit the harvestable yield by reducing quality and marketability (Andrews & Powell 2009).

In its native range in eastern North America, WAA overwinters as eggs on its primary host, the elm tree *Ulmus americana* L (Baker 1915). Although sexual reproduction does sometimes occur (Sandanayaka & Bus, 2005), its life cycle is often considered as anholocyclic (without sexual reproduction) in most regions of the world where its primary host is absent (Beers *et al*. 2010). Thus, overwintering occurs as adult females on both below and above ground tissues in regions like Australia (Nicholas *et al*. 2005) and new infestations from early spring typically start with young nymphs produced from overwintering adults. These adults were either overwintering on the roots or were concealed within protected aerial plant tissues such as bark crevices and galls (Nicholas *et al*. 2005; Beers *et al*. 2010). Once colonies have been established on aerial parts, further dispersal of nymphs by wind or human activity allows colonisation of new hosts.

During the last decade, this pest insect has re-emerged as an important issue in North America (Beers *et al*. 2010), Europe (Lemoine & Huberdeau 1999), South Africa (Timm *et al*. 2005), Australia (Nicholas *et al*. 2005; Andrews & Powell 2009) and New Zealand (Rogers *et al*. 2011), causing reduced productivity of apple orchards. This pest resurgence is attributed to three main factors: Firstly, some insecticides are no longer used either because they do not meet new legislative requirements, have been withdrawn, or because they can affect survival of natural or introduced enemies (Lemoine & Huberdeau 1999). This is the case with organophosphates (e.g. chloropyrifos and diazinon) and carbaryl (Cohen *et al*. 1996; Bradley *et al*. 1997; Rogers *et al*. 2011). Secondly, the selection of dwarf rootstocks (e.g. M9 and M26) for higher productivity but with limited resistance against WAA colonisation (Beers *et al*. 2010). Thirdly, WAA can overcome the resistance provided by some rootstocks (e.g. MM106 and M793, McClintock 1930; Giliomee *et al*. 1968). These rootstocks are typically used by apple growers to prevent infestations of the root system, and previous research has identified genes, such as *Er1* or *Er2,* that are involved in the rootstock resistance mechanism against WAA (Bus *et al*. 2008).

Since it was first reported in Australia in the 19th century (Nicholls 1919), WAA has become widespread and several populations that are geographically isolated may have evolved into different biotypes (Claridge & Den Hollander 1983). Each WAA biotype could have a different interaction with the plant host, leading to differences in behaviour, fecundity, and growth rate. This could affect the efficacy of natural or exotic enemies that could be used in an Integrated Pest Management (IPM) approach. WAA biotypes exist in USA (Rock & Zeiger 1974; Young *et al*. 1982), South Africa (Giliomee *et al*. 1968), New Zealand (Sandanayaka *et al*. 2003) and Australia (Sen Gupta & Miles 1975; Andrews & Powell 2009; Costa et al. 2014). WAA is often held in check by biocontrol agents such as the parasitoid wasp *Aphelinus mali* and earwigs (Nicholas et al. 2005).

Recent reports suggest that growers may be reverting to full pesticide programs, despite the threat of resistance developing against newer pesticides being put under increased selection pressure. Recent incursions of Q-fly caused quarantine authorities to withdraw support for Q-fly SIT and issue advice that includes spraying of pesticides. Such spraying is likely to severely disrupt current IPM programs. Several projects are being developed to find alternatives to spraying fruit flies. These include the use of SIT (Sterile Insect Technique), improved baiting and trapping, and area-wide management approaches. The apple and pear industry still has a goal of reducing pesticide usage to ensure market access is protected. The release of CM parasitoid *Mastrus ridens* as an output of the recently completed first phase of the PIPS program, combined with mass-trapping of CM females and the use of MD will potentially avoid the use of insecticide cover sprays against CM, and provide effective biocontrol of CM in pome fruit.

Proof of concept for mass-trapping of codling moth using enhanced lures has now been successfully completed. The new lures attract both sexes of codling moth. The research utilised 43 traps/ha, but results suggest that 3-8 traps/ha may be sufficient to control codling moth in orchards where MD is also being used. Trap placement applied in this way has the advantage of giving growers a good indication of both moth population levels and location of hotspots in the orchard.

Summary

Mass-trapping of moths that survive attack by *Mastrus* will reduce populations of both sexes and therefore improve the performance of mating disruption and reduce the number of eggs being laid. Ovicides could then be used to kill eggs before they hatch, and codling moth granulosis virus could be sprayed to kill caterpillars that survive the ovicides. Incorporating MD when necessary against OFM, and encouraging biological control agents against other pests such as WAA, LBAM (lightbrown apple moth, *Epiphyas postvittana* Walker), and mites, will provide a suite of "soft" techniques that should reduce the risks associated with chemical residues.

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